CONFERENCE ON OILSEED PROTEINS

Characteristics and Food Industry Requirements

New Orleans, Louisiana
November 6-7, 1972

Southern Regional Research Laboratory
Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

FOREWORD

In recent years increasing interest in improving the nutritive quality of our diet and efforts to supply needed protein to larger areas of the world have focused attention on oilseed proteins. Dr. Harold L. Wilcke originally suggested the need for a conference on the characteristics of oilseed proteins that would provide an interface between research workers in industry and those in government and academic institutions. As a result, and with the encouragement of Dr. C. H. Fisher, ARS retired, and Dr. Fred R. Senti this conference was undertaken.

The conference "Oilseed Proteins--Characteristics and Food Industry Requirements" sponsored by the Southern Regional Research Laboratory was organized with the stated objectives of determining:

1. What properties does the food industry want in oilseed protein products?

2. What is known about the properties of oilseed protein products and their variants?

3. What research and products are needed to successfully integrate oilseed proteins into the food supply chain?

The program presented here was organized with the aid of a program planning committee drawn in part from outside the SRRL. We are grateful for the enthusiastic response and the guidance of the discussion leaders and speakers. However, this conference can be truly successful only with the active participation of everyone in attendance. With your cooperation it is hoped that at the end of the conference we will be better able to identify the research and product needs for the successful integration of oilseed proteins into the food supply chain.

H. L. Wilcke, Ralston Purina Co. (Honorary Chairman)
R. J. Dimler, NRRL
W. H. Martinez, SRRL
R. L. Ory, SRRL
J. L. Pence, WRRL
K. J. Smith, NCPA
M. P. Thompson, ERRL
H. L. E. Vix, SRRL

L. A. Goldblatt, SRRL (Chairman)

PROGRAM

Conference on Oilseed Proteins Characteristics and Food Industry Requirements

November 6-7, 1972
Southern Regional Research Laboratory
New Orleans, Louisiana

Tuesday Morning, November 7 Page

Identification of Common Requirements and Methodologies

9:00-11:00 Group A Discussions
Chairman - Max Milner, Director, PAG, United Nations

Session Reports

A-1 Cereal Systems--K. A. Gilles
A-2 Meat Systems--J. Rakosky, Jr.
A-3 Milk Systems--M. J. Pallansch
General Discussion

Selected Aspects of Functionality

9:00-12:00 Group B Discussions
Chairman - M. P. Thompson, ERRL

Fat Emulsification

9:00 Determination of Emulsification Capacity
R. L. Saffle, University of Georgia 102
Relationship Between Determination and Actuality
R. G. Cassens, University of Wisconsin 104
Comments and Discussion

Water in Food Systems

10:45 Unfreezable Water in Food Systems--Its Determination and Significance in Relation to Other Properties of Absorbed Water
R. B. Duckworth, University of Strathclyde, Scotland 110
Water Activity and Relationship to Functionality
T. P. Labuza, University of Minnesota 112
Comments and Discussion

Tuesday Afternoon, November 7

2:00-4:00 General Session
Chairman - L. A. Goldblatt, SRRL

Report of Group A - Max Milner
Report of Group B - M. P. Thompson
General Discussion

Summary and Close -- H. L. Wilcke

Conference Objectives

by H. L. Wilcke

This conference, it seems to me, is unique in that we are not concentrating on the presentation of results of research that has been accomplished, but rather are trying to achieve some consensus on the type of proteins which may not only be useful, but which are needed in the production of our food systems and also to provide guidelines for the development of criteria for the evaluation of those proteins. It is hoped too that in this conference both the adequacies and inadequacies of methodology available to us for the evaluation of these proteins may be clearly identified.

If we are to meet with any real success in attaining these objectives, we must have free, open, and frank discussions. This does not mean that anyone will be asked or expected to divulge anything that is of a confidential nature to his company or to his laboratory. It does mean that some of us perhaps will have to be more realistic in what we consider to be "classified" information. It certainly means that problems that are common to many should be brought out into the open for discussion, and in some cases a natural reticence to discuss problems must be subjugated to a consideration of the possibility of benefits to be derived from open discussion. We are quite certain that the people who have been invited to this conference are capable of making such decisions in the best interest of everyone concerned.

The rationale for this conference really resulted from the realization of the Technical Advisory Committee to the work at this laboratory that they could not, with any degree of confidence, answer the question as to the relative importance of the standard criteria that have been used for the evaluation of protein isolates. As a result, a questionnaire was prepared and sent out and many of you were gracious enough to respond to that questionnaire.

However, when the results were tabulated, and there was a very good return on the results, some very pertinent questions arose. Is it true that only 25% of the food companies are interested in most of the functional characteristics which have been used to evaluate proteins in the past? Is the ability to form a stable foam of interest to only about 25%? Is the extrudability,the spinnability, or the compatability of interest to less than 25% of the responders? Is heat coagulation of interest to only about 60%? Does this mean that the food companies concerned expect to obtain this information for themselves, and that these particular characteristics are not important to them in deciding whether to evaluate a protein product in the first place?

All of these questions, and many more that we could ask, leave the research people at the bench with very nebulous ideas of just how far they should or must go in developing new types of products before the food companies would be interested in looking at them. There is also the question of terminology. Should we continue to use the terminology of flours, concentrates and isolates? Do we need new terminology? Should we continue to use present terminology with expansion, including new terms? Or do we want to abandon the present terminology and use entirely new methods of designating the products that are desired?

In order to arrive at reasonable and logical answers to these types of questions, two classes of participants have been invited. One we may classify

as the users or potential users of protein products in food systems. The second group would be those who are involved in research and development processes and whose work will result in the type of products that will be offered to the food industry. We have been quite fortunate in getting the acceptance of two speakers who have broad knowledge of the need and possibility for protein products in food systems and also in the type of products that are available today. We have asked these two speakers to put the problems before us in proper perspective. After they have completed their presentations, it will not be appropriate to discuss the relative merits of sources of protein except in that they may be used as examples to illustrate a point.

If we are to achieve our objectives, we must separate the thinking of crude sources such as the soybean, the cottonseed, the peanut, the milk, etc., and focus the consideration on the protein itself. We are not concerned here with the question of whether protein from the soybean may have a flavor problem or the protein from cottonseed may have a color problem. We are concerned with whether flavor or color are important in the product that is presented to the food industry. We are not concerned at this conference in how to utilize the products that are presently available on the market. We are concerned with learning of the particular types of products that the food industry desires. We would hope that the discussions will not be limited to what we think might be possible at the present time. Certainly, no good purpose would be served by ideas that are completely "blue sky," but if there is a need for types of products that are not currently available, certainly they should be noted. We are confident that the type of people invited to this conference will come up with challenges to the research and development people, but that at the same time they will set reasonable and attainable goals.

The conference has been so organized that immediately following lunch we will split into two groups. The group that has been designated A may be described as the users, and Group B which will be the research and development group. The general subject of the discussion of Group A will be concerned with the properties that food companies require. This group will be divided into three sub-groups, the first of which will discuss bakery goods, breakfast foods, pasta, baby foods, and soups that fit into the general classification of cereals. Each of these areas will have a discussion leader, and all of the participants in each sub-group will be expected to participate in the discussion of each of these subjects.

The objective will be to determine the types of protein products needed in each of these areas and the criteria for their evaluation. When this has been accomplished for each of the five areas, the sub-group will attempt to consolidate their discussions to come up with the least possible number of products that will suit their needs and also the criteria that will be needed.

Sub-group 2 will discuss meat products, baby foods, and those food products which incorporate meat-type components. They will follow the same procedure as sub-group one, as will sub-group 3, which will discuss beverages, desserts and toppings, coffee whiteners, and baby foods which fit into the liquid classification.

We have asked K. A. Gilles of North Dakota State University to assume the chairmanship of the five areas in sub-group one, Joe Rakosky of Central Soya for sub-group two, and Mike Pallansch of U.S.D.A. for sub-group three.

The afternoon has been allotted for this type of discussion and we hope that this will be ample time for this phase of your deliberations. However, if more time is required, the discussions may be continued tomorrow morning.

Tomorrow morning there will be a general discussion when all three of the sub-groups meet as a whole to form group A. At this time the three sub-groups will try to condense the ideas they have had to come up with a final list of products and criteria for their evaluation. If any of the sub-groups complete their deliberations ahead of schedule, they are perfectly free to attend the discussion of other sub-groups or of group B.

Group B will not be subdivided. This group will discuss factors affecting characteristics of protein products. This afternoon they are scheduled to discuss cellular structure of oil seeds and protein composition and configuration.

A special session has been set up this evening for the purpose of discussing the relationship of composition to functionality. All participants are invited to attend this session and participate if they so desire. Tomorrow morning group B will discuss selected aspects of functionality. Following these discussions then, there will be a preparation of reports under the leadership of Dr. Max Milner for group A and Dr. Marvin Thompson for group B. These reports will be presented tomorrow afternoon, after which we will have a summary and close. The objectives which we will try to meet in the final report are:

1. To provide guidelines for the research and development functions on the identification, isolation, and development of fractions of oilseeds which have potential value in our food systems.
2. To identify the adequacies as well as the inadequacies and deficiencies which exist in methodology for the evaluation of these proteins.
3. To suggest uniform terminology for the designation of protein products, regardless of the sources from which they are derived.

With this type of information we feel confident that the research and development people will be able to organize their work much more efficiently and economically, both from the standpoint of manpower and funds, and that the food industry will be offered products which are much more in line with their needs and desires.

Table I

METHOD OF PRODUCING SOY FORTIFIED BREAD
BY A 70% SPONGE-DOUGH FORMULA AND PROCEDURE

Sponge:

Soy Fortified Flour*	70%
Water	42%
Yeast	2.5%
Yeast Food	0.5%
Lard	2.0%

Mix three minutes number 1 speed in Hobart A-200 mixer with McDuffee bowl, at 78°C.

Ferment four hours at 83°F.

Dough:

Soy Fortified Flour*	30%
Water	26%
Salt	2.25%
Sugar	4.5%

Mix 2 minutes at number 1 speed, plus 3 minutes number 2 speed; dough temperature, 80°F; floor time, 45 minutes; scale 19 ounces; 10 minutes intermediate proof; mould and pan; panary proof to 1/2 inch above top of pan at 107°F and 85% relative humidity; bake 20 minutes at 435°F.

* Soy fortified flour is composed as follows:

Bread Wheat Flour	88 parts
Soy flour, defatted (lightly toasted)	12 parts
Dough improver (sodium stearoyl-2-lactylate)	0.5% of product

Table II

METHOD OF PRODUCING SOY FORTIFIED
BREAD BY A STRAIGHT DOUGH FORMULA AND PROCEDURE

Soy Fortified Flour*	100%
Water	70%
Yeast	3%
Yeast Food	0.25%
Lard	2.0%
Salt	2.25%
Sugar	4.5%

Mix two minutes at number 1 speed, plus 5 minutes at number 2 speed in Hobart A-200 mixer with McDuffee bowl; dough temperature, 83°F; fermentation time, 45 minutes; scale 19 ounces; 10 minutes intermediate proof, mould and pan; panary proof to 1/2 inch above top of pan at 107°F and 85% relative humidity; bake 20 minutes at 435°F.

* Soy fortified flour is composed as follows:

Bread wheat flour	88 parts
Soy flour, defatted (lightly toasted)	12 parts
Dough improver (sodium stearoyl-2-lactylate)	0.5% of product

Table III

METHOD OF PRODUCING BREAD BY A
100% SPONGE FORMULA AND PROCEDURE

Sponge:

Soy Fortified Flour*	100%
Water	69%
Yeast	2.75%
Yeast Food	0.5%
Lard	2.0%

Mix 3 minutes at number 1 speed in Hobart A-200 mixer with McDuffee bowl, at 83°F.

Ferment one hour at 83°F.

Remix:

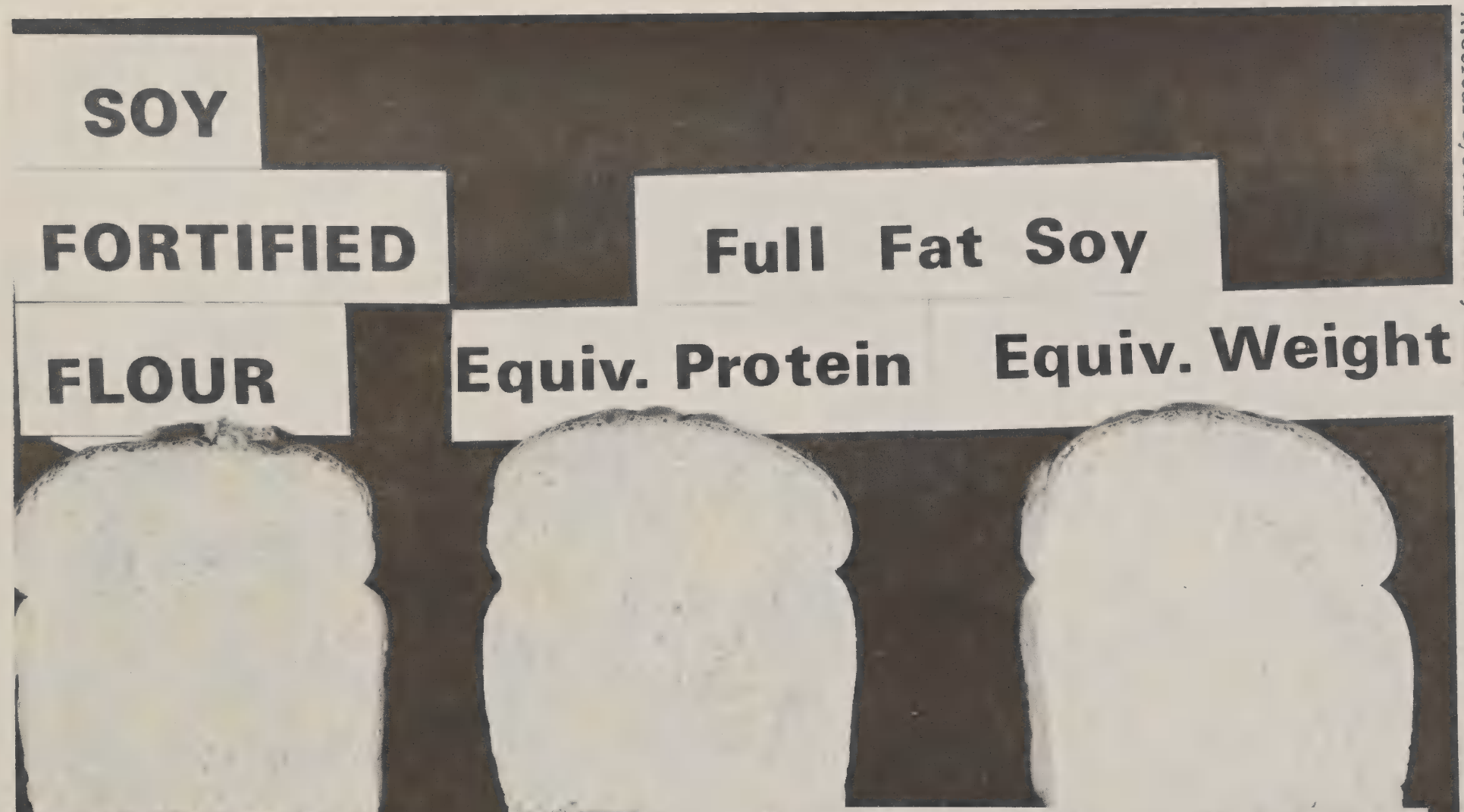
Salt	2.25%
Sugar	4.5%

Mix two minutes at number 1 speed, plus three minutes at number two speed; dough temperature, 83°F; floor time, 45 minutes; scale 19 ounces; 10 minutes intermediate proof, mould and pan; panary proof to 1/2 inch above top of pan at 107°F and 85% relative humidity; bake 20 minutes at 435°F.

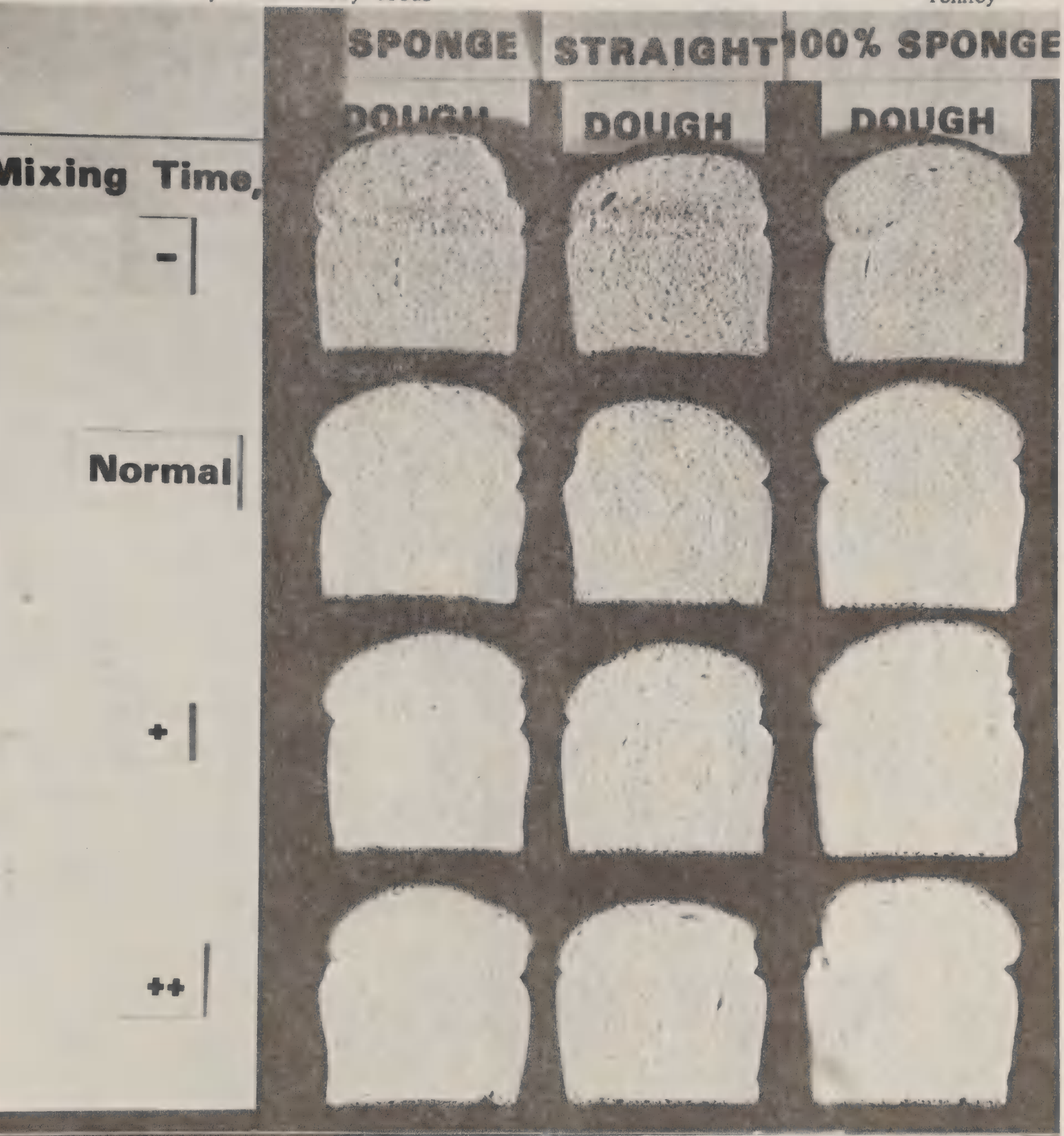
* Soy fortified flour is composed as follows:

Bread Wheat Flour	88 parts
Soy flour, defatted (lightly toasted)	12 parts
Dough improver (sodium stearoyl-2-lactylate)	0.5% of product

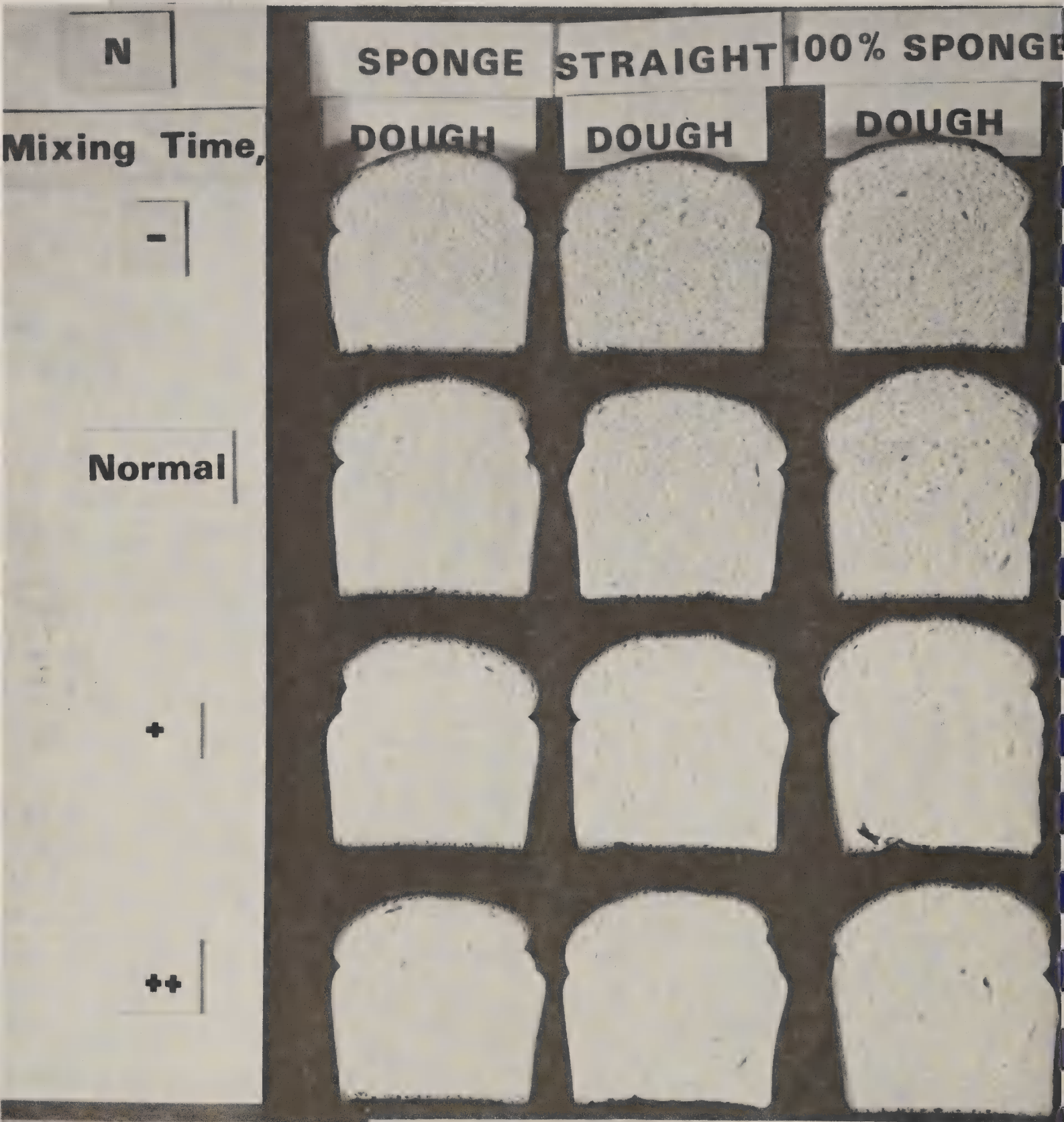
Photograph 1: The relative extensibility of bread doughs prepared from bakers patent flour and from soy fortified flour.



Photograph 2: Comparison of breads prepared from soy fortified flour (defatted soy flour) and full-fat soy flour on an equivalent weight and on an equivalent protein basis.



Photograph 3: Comparison of breads prepared from soy fortified flour (chemically treated, defatted, lightly toasted soy) by three baking methods.



Photograph 4: Comparison of breads prepared from soy fortified flour (non-chemically treated, defatted, lightly toasted soy) by three baking methods.

SOY PROTEIN PRODUCTS FOR VARIOUS MEAT SYSTEMS

	50% PROTEIN			70% PROTEIN DRY BASIS		90% PROTEIN DRY BASIS	
	SOY FLOUR	SOY GRITS	TEXTURED SOY PROTEIN	SOY PROTEIN CONCENTRATE		ISOLATED SOY PROTEIN	SPUN FIBER
				COARSE	FINE		
GROUND							
PATTIES		X	X	X			X
MEAT BALLS		X	X	X			X
MEAT LOAVES		X	X	X			X
CHILI	X	X	X	X			X
SLOPPY JOE		X	X	X			X
TACOS	X	X	X	X			X
SALISBURY STEAK	X	X	X	X			X
SAUSAGE		X	X	X			X
EMULSION							
SAUSAGE	X				X	X	
BOLOGNA	X				X	X	
LOAVES	X				X	X	
CANNED	X				X	X	
OTHER							
BABY FOODS	X	X	X	X	X	X	X
SOUPS							
CANNED	X	X	X	X	X	X	X
DRY			X			X	X
SAUCES & GRAVIES	X	X	X	X	X	X	X
PET FOODS	X	X	X	X	X	X	X

TABLE 1. Protein quality infant formula.

Protein	Adjusted PER*
Defatted soy flour	1.65
Acid washed soy flour	1.93
Soy protein isolate	1.25
Soy protein + .5% Met.	1.72
Soy protein + 1.0% Met.	1.87

Weanling rats - diet supplied 10% protein, 20% fat and ample minerals and vitamins.

*Adjusted to casein at PER = 2.5

Source: 24-030

TABLE 2. Protein quality (PER) related to methionine supplementation and type of sterilization.*

Protein	Unheated	Conventional In-Can Sterilization	Aseptic Canning
Soy Protein Isolate....	.55-.93	1.4	1.3
Soy Protein Isolate + 0.5% Met.....	--	1.9	1.9
Soy Protein Isolate + 1.0% Met.....	--	1.9	1.9

Weanling rats; diet supplied 10% protein, 20% fat and ample minerals and vitamins.

*Adjusted to casein at PER = 2.5.

Source: 24-057

TABLE 3. Protein quality (PER and NPR) related to source of soy protein.

Protein	Adjusted PER	Percent of Casein	7-Day NPR	Percent of Casein
Soy Protein Isolate				
A plus methionine.....	2.0	82	3.50	66
B plus methionine.....	2.3	90	4.71	89
D plus methionine.....	2.1	85	4.06	77
Soy Flour				
E	1.5	60	3.53	67
Casein				
C	2.5		5.80	

Weanling rats; 10% protein, 20% fat diet.

Source: 24-082

TABLE 4. Effect of heating on PER as related to trypsin inhibitor content of several legumes.

Improved by Autoclaving		Not Improved by Autoclaving	
Trypsin Inhibitor Present	Trypsin Inhibitor Absent	Trypsin Inhibitor Present	Trypsin Inhibitor Absent
Lima bean	Jack bean	Peanut	Guar bean
Common bean	Lentil	Chick pea	Common pea
Soy bean	Horse bean	Sweet pea	
Blackeye pea (cowpea)		Mung bean	

Source: Borchers, R. and Ackerson, C.W., J. Nutrition 41: 339,1950

TABLE 5. Essential amino acids of some protein foods, compared with cow milk at 100.

Food	Iso.	Leu.	Lys.	Phe.	Cys+ Met.	Met.	Thr.	Try.	Tyr.	Val.
Oil Seeds (1)										
Cottonseed	58	59	54	105	90	56	76	82	53	70
Peanut.....	63	60	45	102	71	36	58	77	69	71
Sesame.....	64	73	32	129	147	114	66	102	78	55
Soy	83	77	80	99	92	55	84	96	63	75
Sunflower.....	72	63	40	90	99	66	72	88	46	71
Animal Protein Foods (1)										
Human milk.....	101	91	81	96	130	91	99	118	110	95
Egg.....	103	90	80	118	162	127	106	118	85	105
FAO Provisional Pattern (2)										
mg AA/g N in edible portion of food	270	306	270	180	270	144	180	90	180	270
Percent of milk protein...	41	49	54	58	128	94	62	100	56	61

(1) Modified data from Teply & Gyorgy, J. Ped. 61:925, 1962.

(2) Protein Requirements, Rome, 1957, Food and Agr. Org. of the United Nations.

TABLE 6. Essential amino acids of some protein foods compared with Fomon's Requirements for infants as 100[1].

Food	His.	Iso.	Leu.	Lys.	Phe.	Met.	Cys.	Thr.	Try.	Val.
Oilseeds[2]										
Coconut	206	178	131	98	198	200	177	142	137	167
Cottonseed	246	149	116	111	240	156	185	157	195	156
Peanut	238	163	119	92	232	100	171	119	182	157
Sesame	247	165	144	66	294	318	247	138	240	123
Soy	257	213	151	164	227	153	202	175	226	166
Sunflower	262	196	125	83	207	185	195	149	208	157
Animal Protein Foods[2]										
Cow milk	269	258	197	206	229	280	104	207	237	222
Human milk	255	260	179	167	218	254	244	205	279	212
Egg	245	271	177	164	271	356	265	220	279	232
Fomon's Provisional Pattern										
mg AA/g N[3]	62	158	315	241	136	57	55	141	38	198
mg AA/100 Kcal	26	66	132	101	57	47	24	59	16	83

(1) Fomon, et.al., 1972, in press, Acta Paediat. Scand.

(2) Modified data from Teply and Gyorgy, J. Ped. 61: 925, 1962.

(3) Based on formula with 67.6 cal. and 1.8 g. protein per 100 ml.

LIST OF ILLUSTRATIONS

B.Cellular Structure-Transmission Electron Microscope Dieckert

Figure 6. Dictyosomes of the peanut embryo.

a. Dense granules in the peripheral region of the dictyosome (D) stains differently from spherosomes (S). Stained with aqueous uranyl acetate. X 56,600.

b. Dictyosome (D) with dense granule (G_1). Also note dense granules (G_2 and G_3) are inside vacuole (V). Stained with alcoholic uranyl acetate. X 56,600.

c. Dictyosome in section coplanar with sacule. Dense granules (G) in the peripheral region of the dictyosome. Rough endoplasmic reticulum in close proximity to dictyosome. Stained with aqueous uranyl acetate. All sections stained with lead citrate. X 56,600.

Figure 7. Endoplasmic reticulum of the cells of a peanut embryo filling with aleurone vacuoles.

a. Dense granules (G) in sacules of the rough endoplasmic reticulum (RER). X 43,300.

b. Same, with mitochondrion (M) and spherosome (S). X 43,400.

c. Section coplanar with the membrane of the rough endoplasmic reticulum, showing the polyribosomes (P). X 54,900.

d. Cross section of the rough endoplasmic reticulum (RER) showing stacks of sacules. X 54,900.

Figure 8. Micro double diffusion analysis of mature aleurone grains and developing embryos of the peanut.

a. Analysis of fractions 8, 44-45, 50, 59, 86 and 96 from DEAE cellulose column with rabbit antiserum against an extract of mature aleurone grains (A/T).

b. Analysis of the same fractions with rabbit antiserum against α-conarachin, (A/α-c).

c. Analysis of an extract (E_1) of 2-5 mm embryos with A/T. The extract (T) of mature aleurone grains served as reference; a non-immune serum (N) and the buffer (B) served as controls.

d. Analysis of an extract (E_2) of 6-8 mm embryos with A/T.

e. Analysis of an extract (E_3) of 10-13 mm embryos with A/T.

f. Analysis of an extract (E_4) of 14-19 mm embryos with A/T.

Figure 9. Chromatography of an extract of aleurone grains on a DEAE cellulose column. Solvent for the extraction and column chromatography was 0.05 M Tris·HCl, pH 7.8, 0.5 M sucrose, 0.015% NaN_3. The proteins were eluted with a linear 0.0 - 0.5 M NaCl gradient at 0-4° C.

Figure 10. Cell of a hypothetical oil seed producing aleurins and sequestering them in a vacuole. The aleurins are synthesized on the polyribosomes (P) of the rough endoplasmic reticulum (ER), concentrated by the dictyosome (D) in membrane-bound vesicles (GV). The aleurins are transferred from the vesicles to the vacuole (V) by membrane fusion. Such cells also contain plastids (L), mitochondria (M) and spherosomes (S) and are encased in a cell wall penetrated by plasmodesmata (PD).

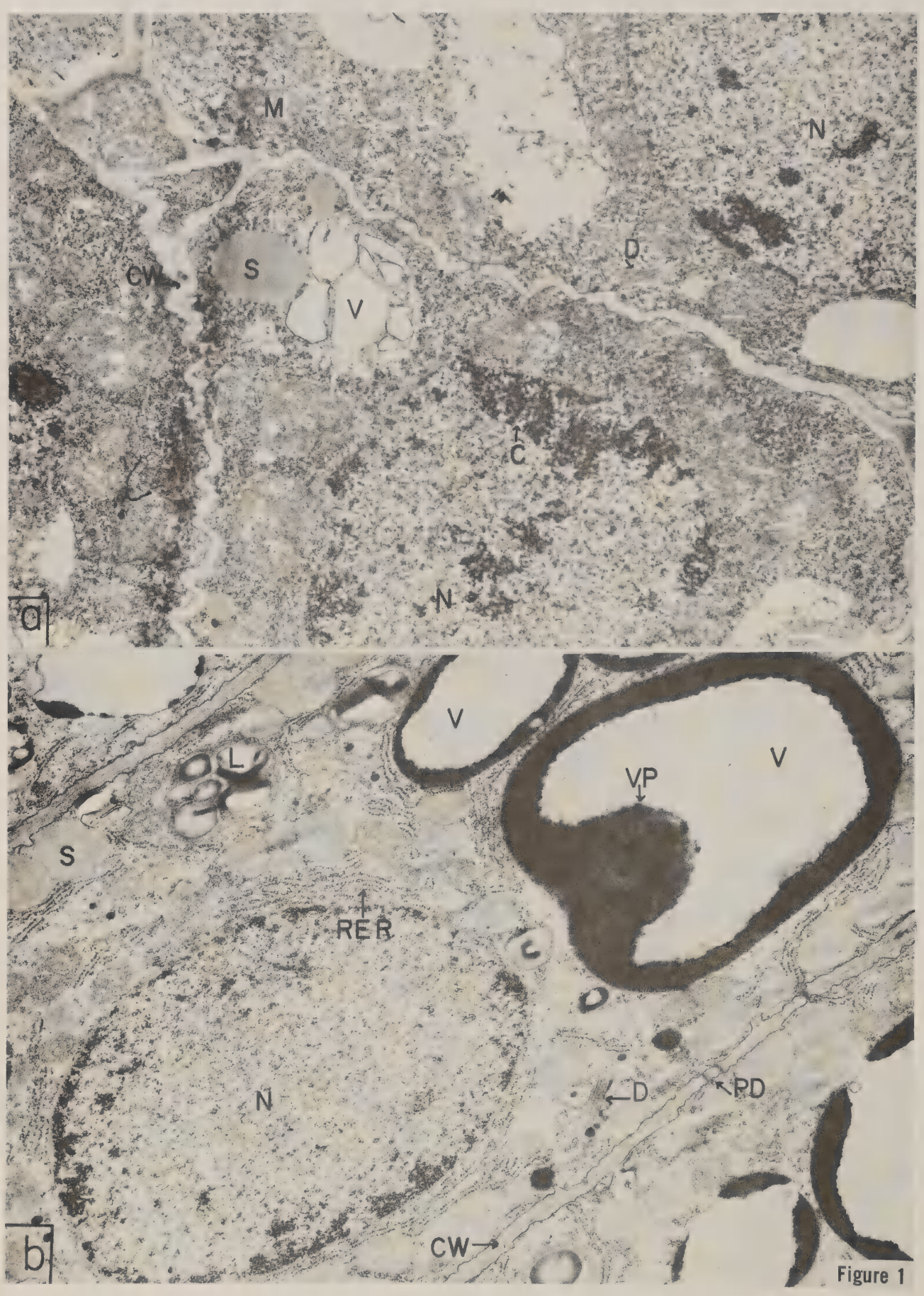


Figure 1

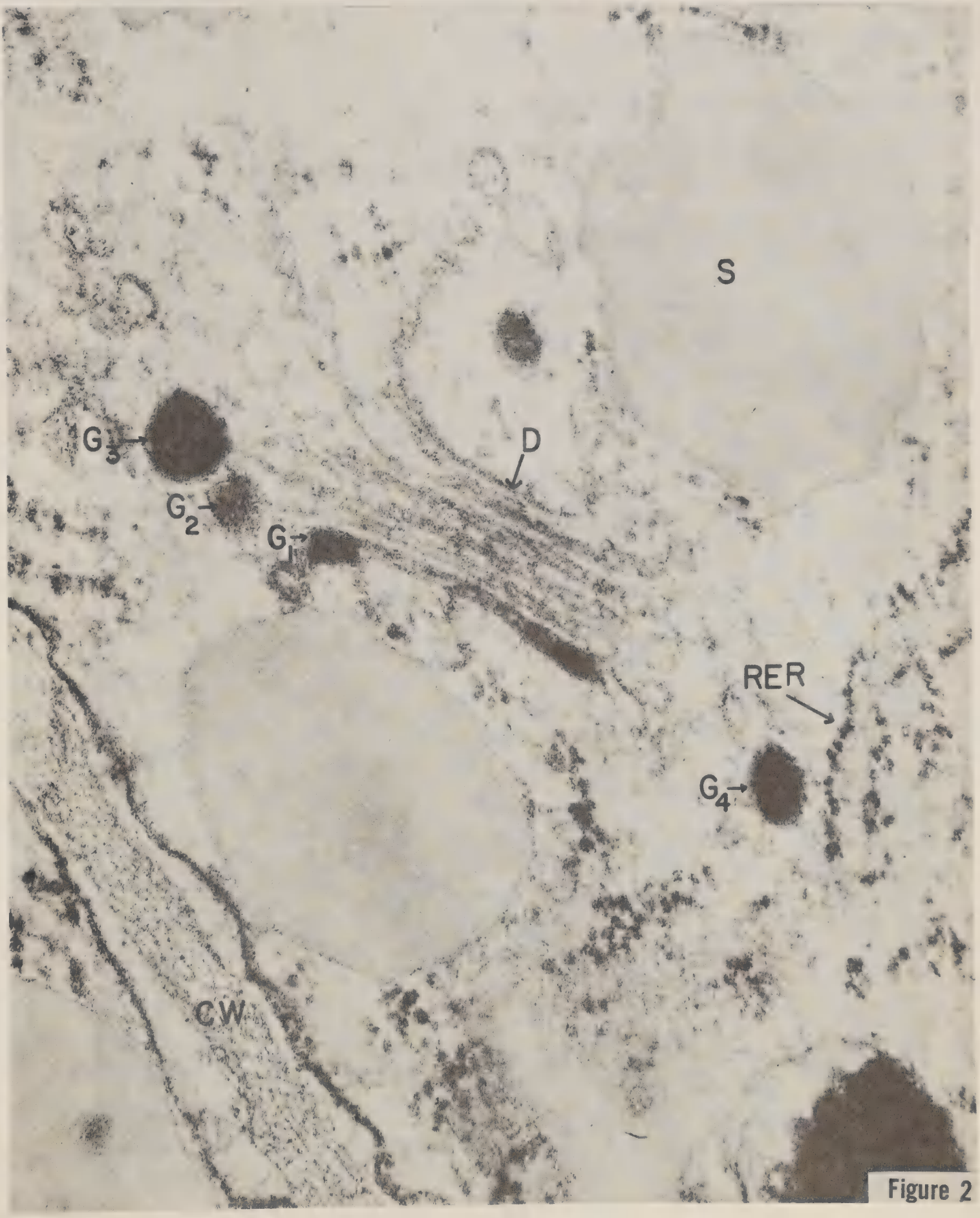


Figure 2

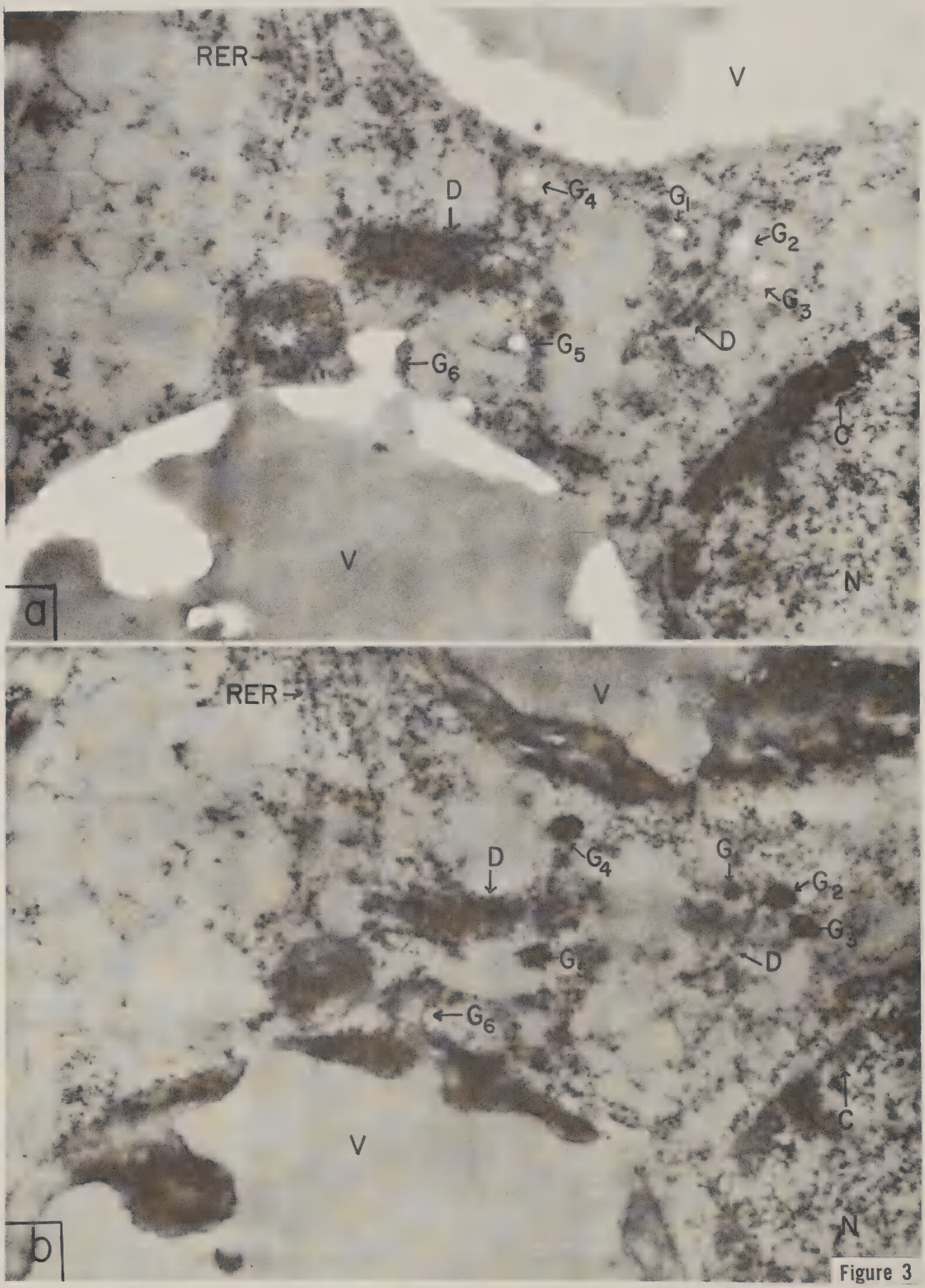


Figure 3

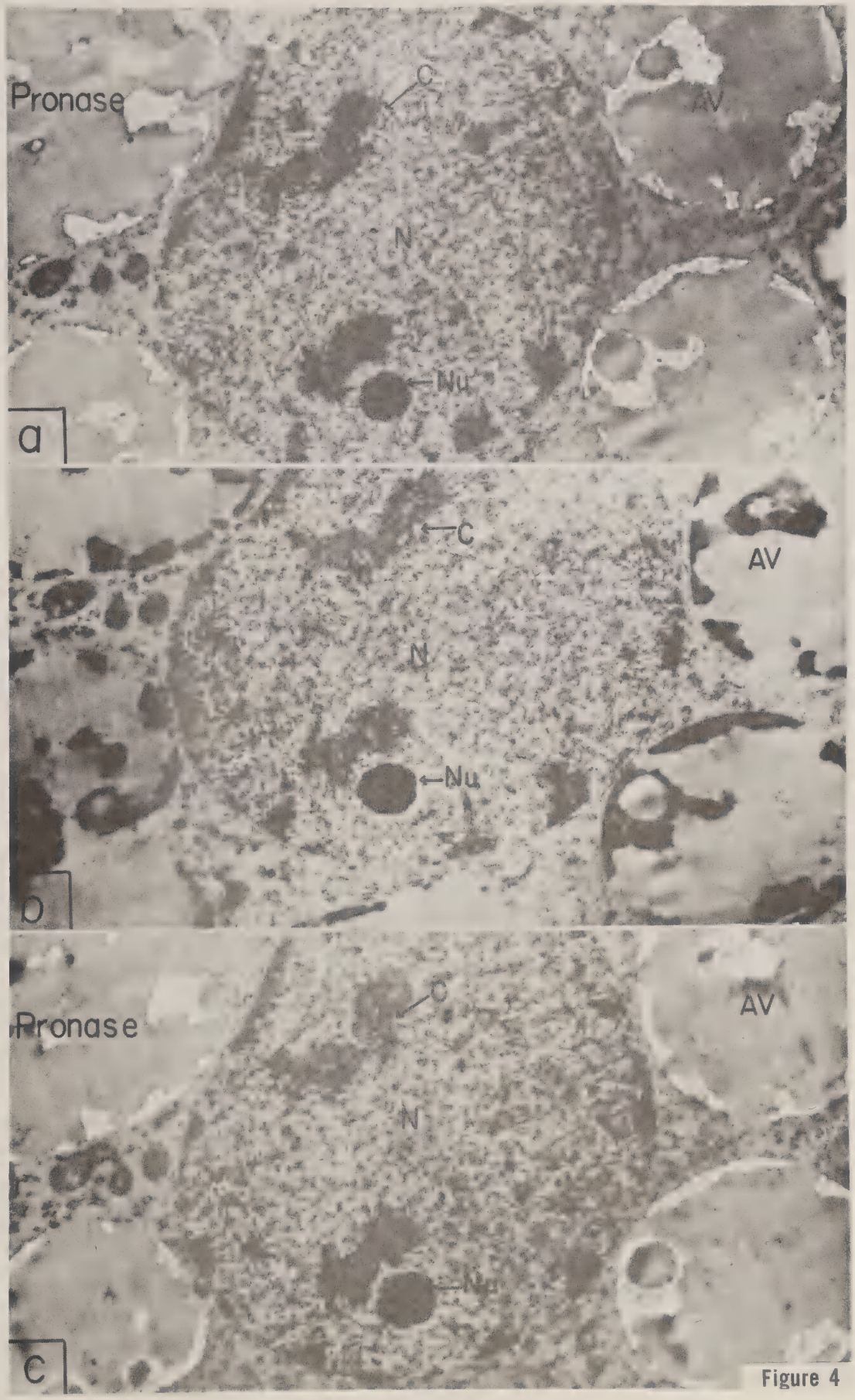


Figure 4

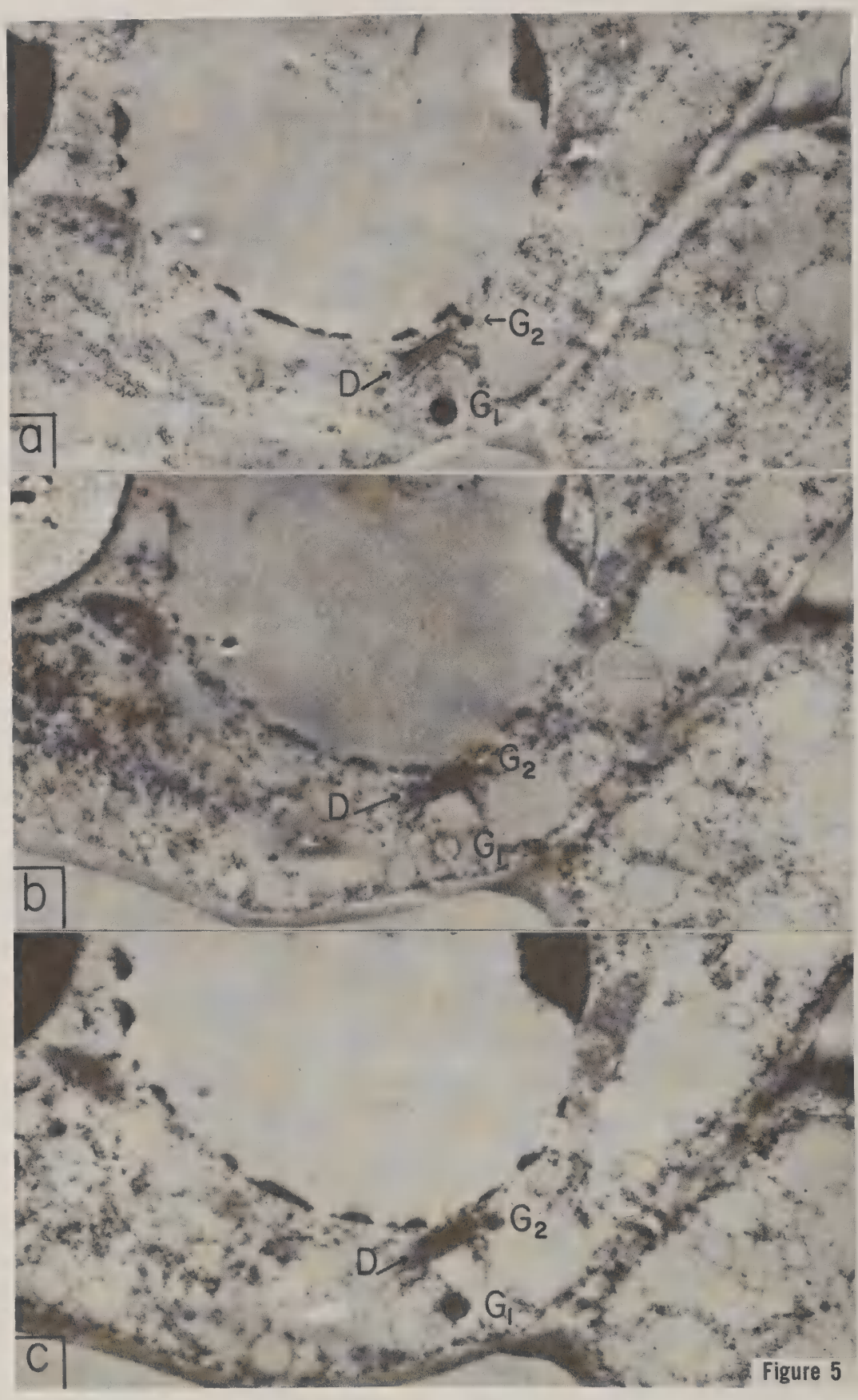


Figure 5

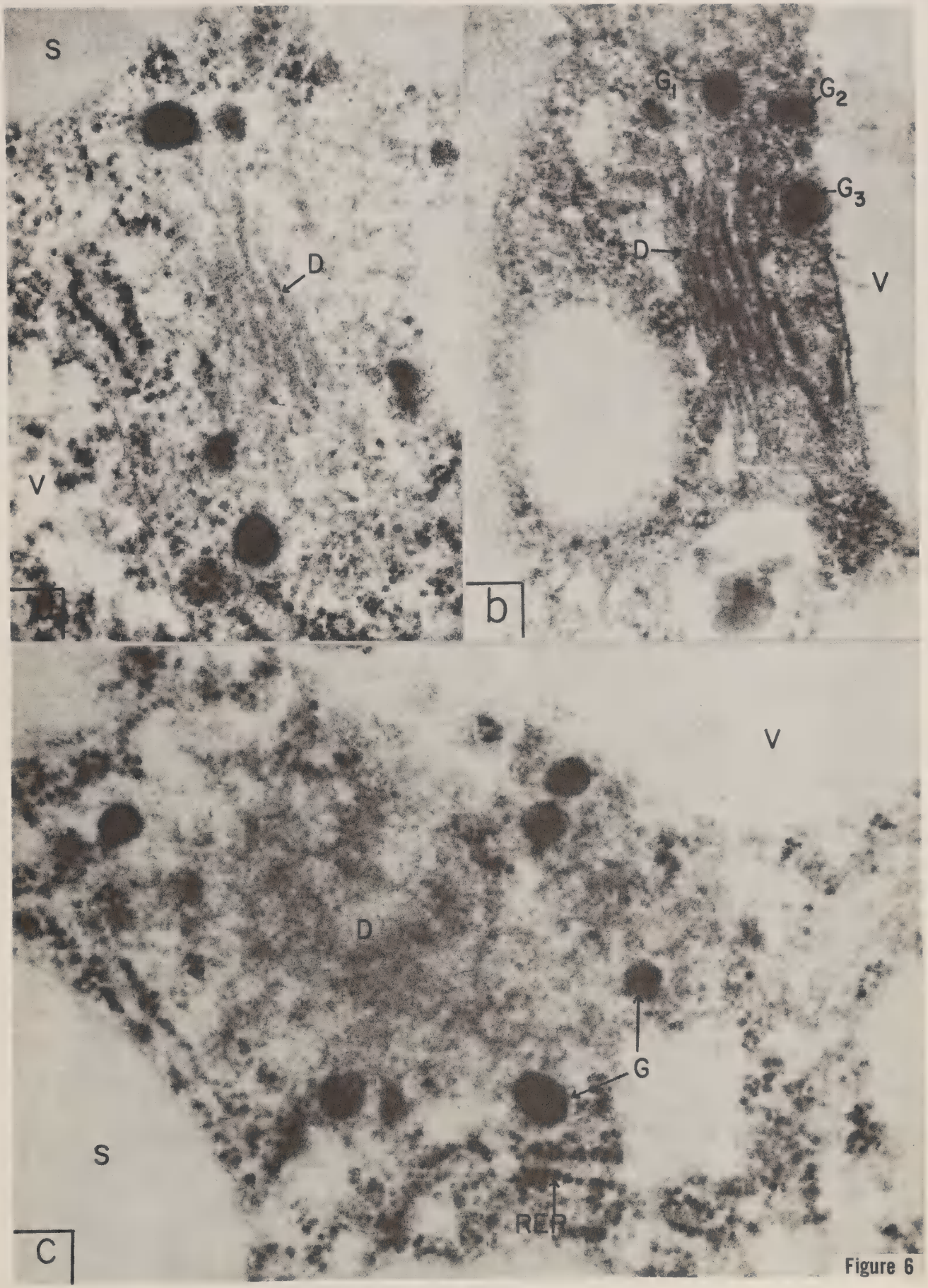


Figure 6

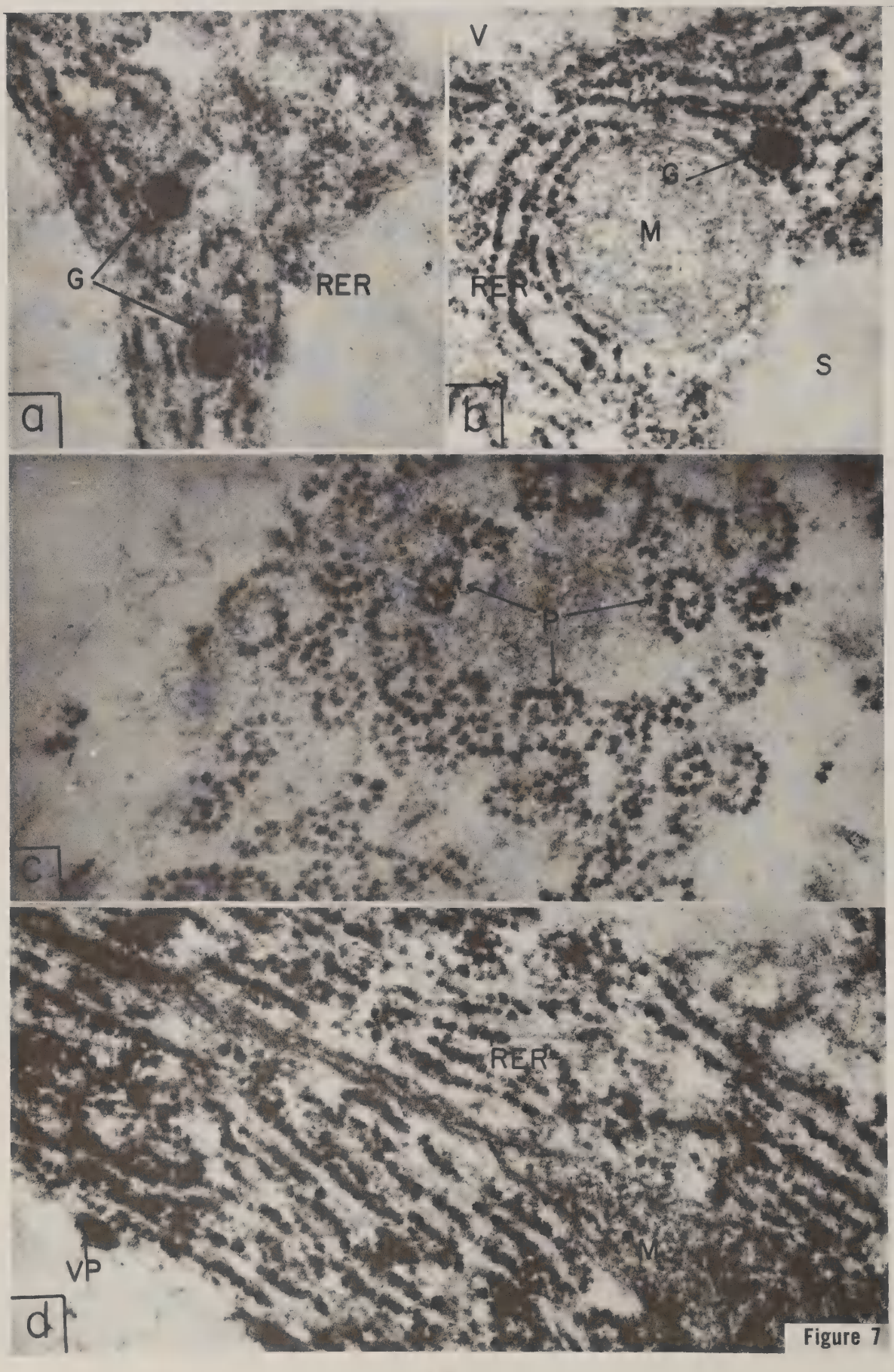


Figure 7

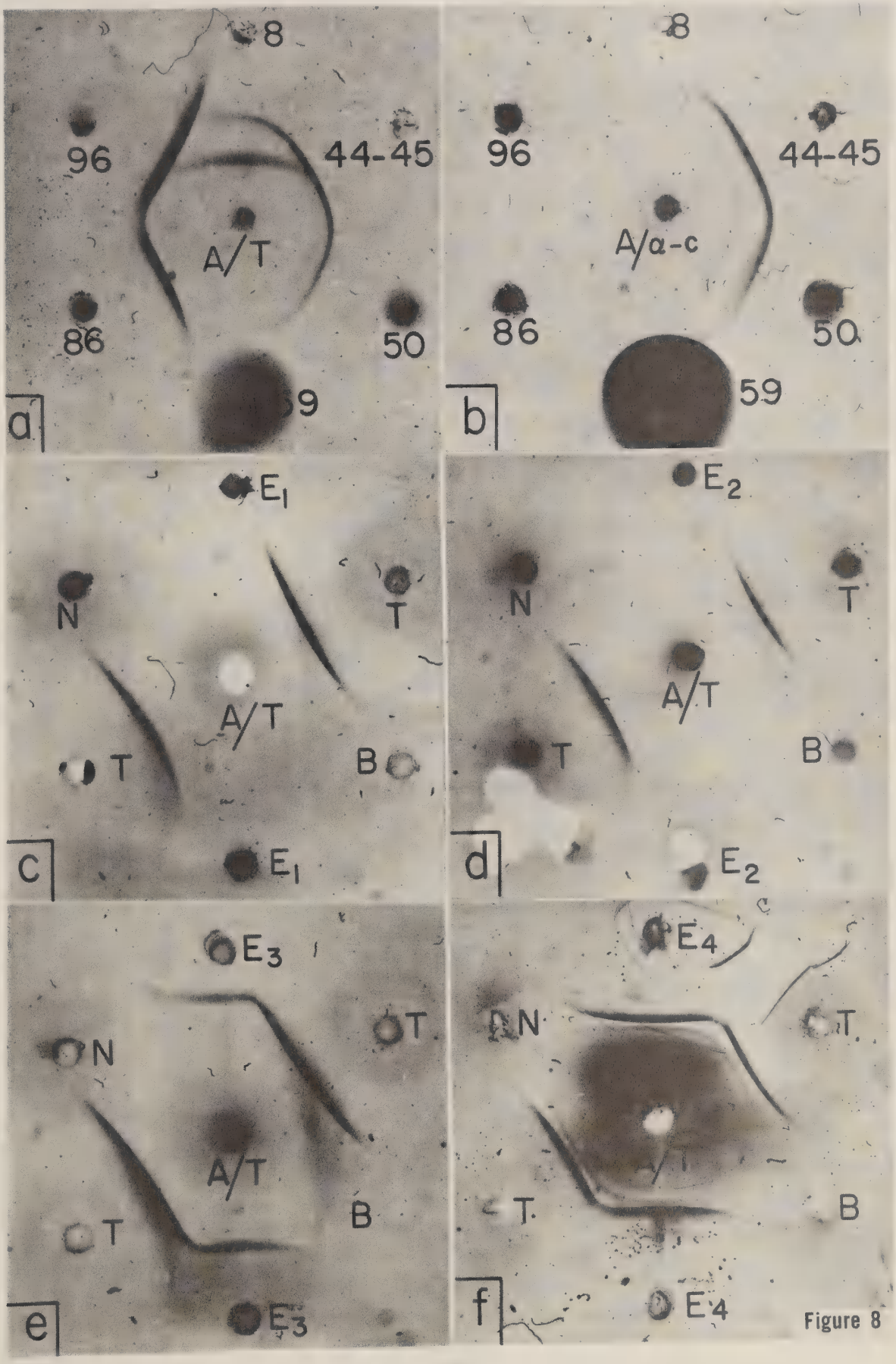

8
96
44-45
A/T
86
50
9
a
8
96
44-45
A/α-c
86
50
59
b
E1
N
T
A/T
T
B
E1
c
E2
N
T
A/T
T
B
E2
d
E3
T
N
A/T
B
T
E3
e
E4
N
T
T
B
E4
f

Figure 8

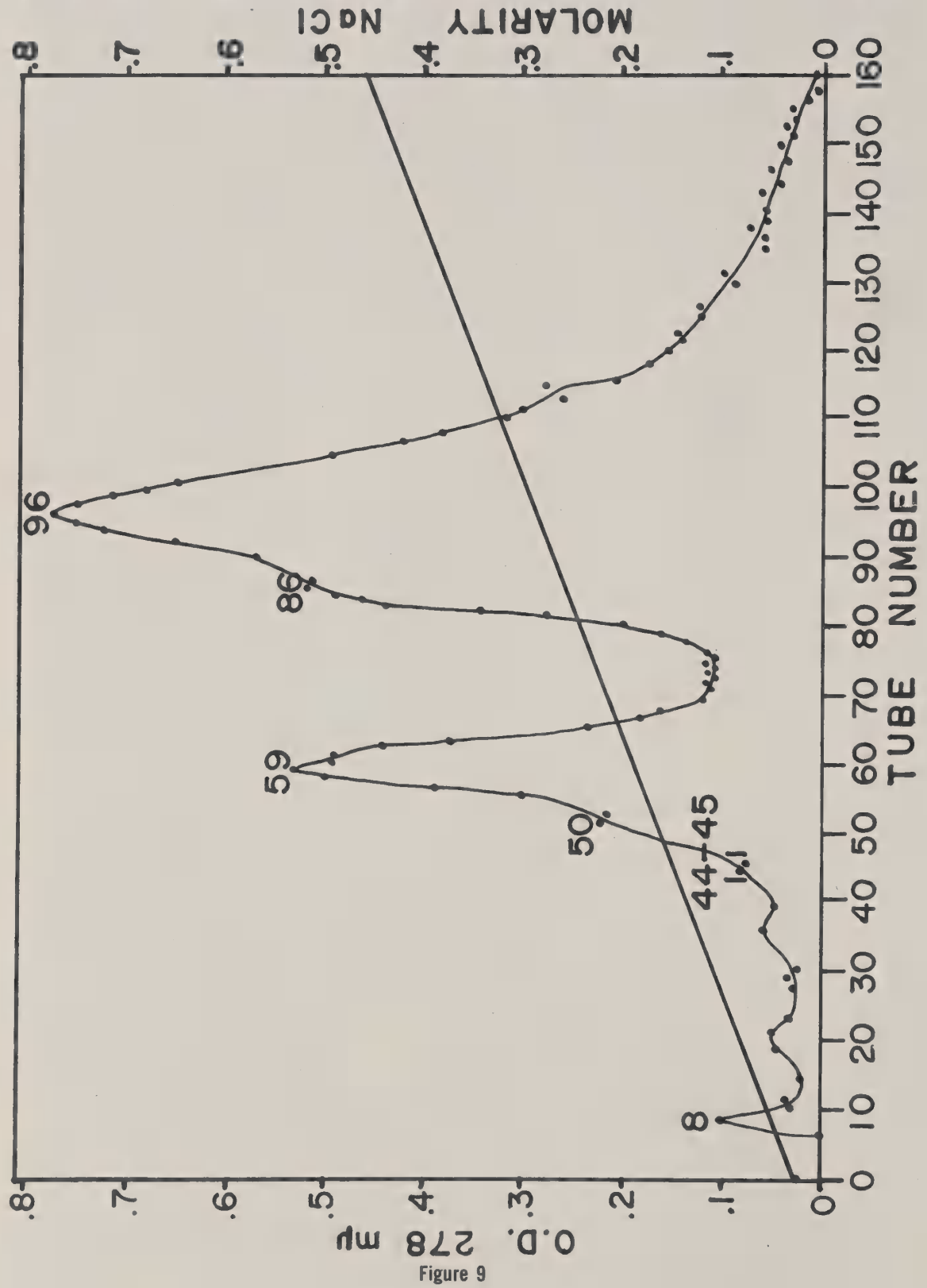


Figure 9

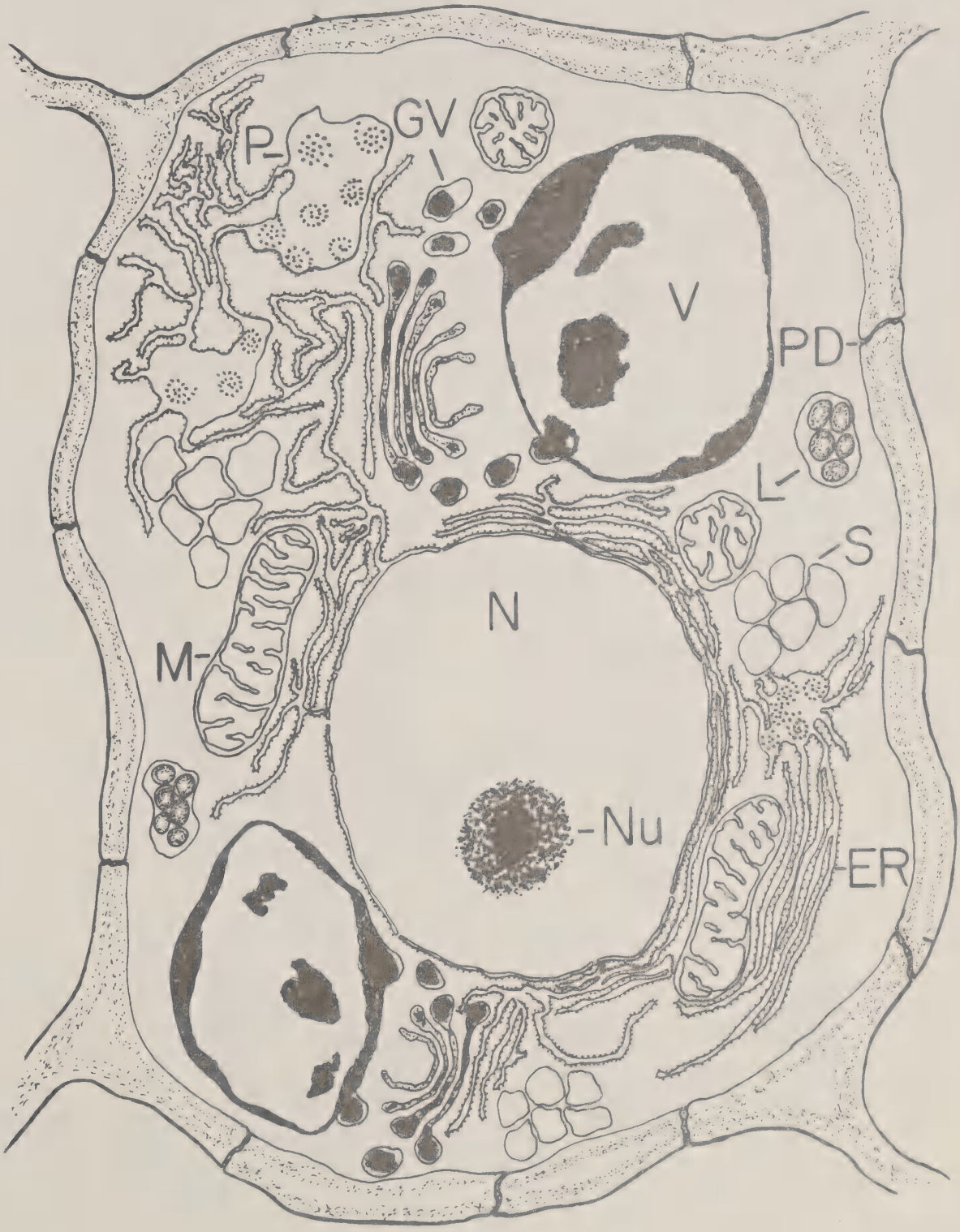
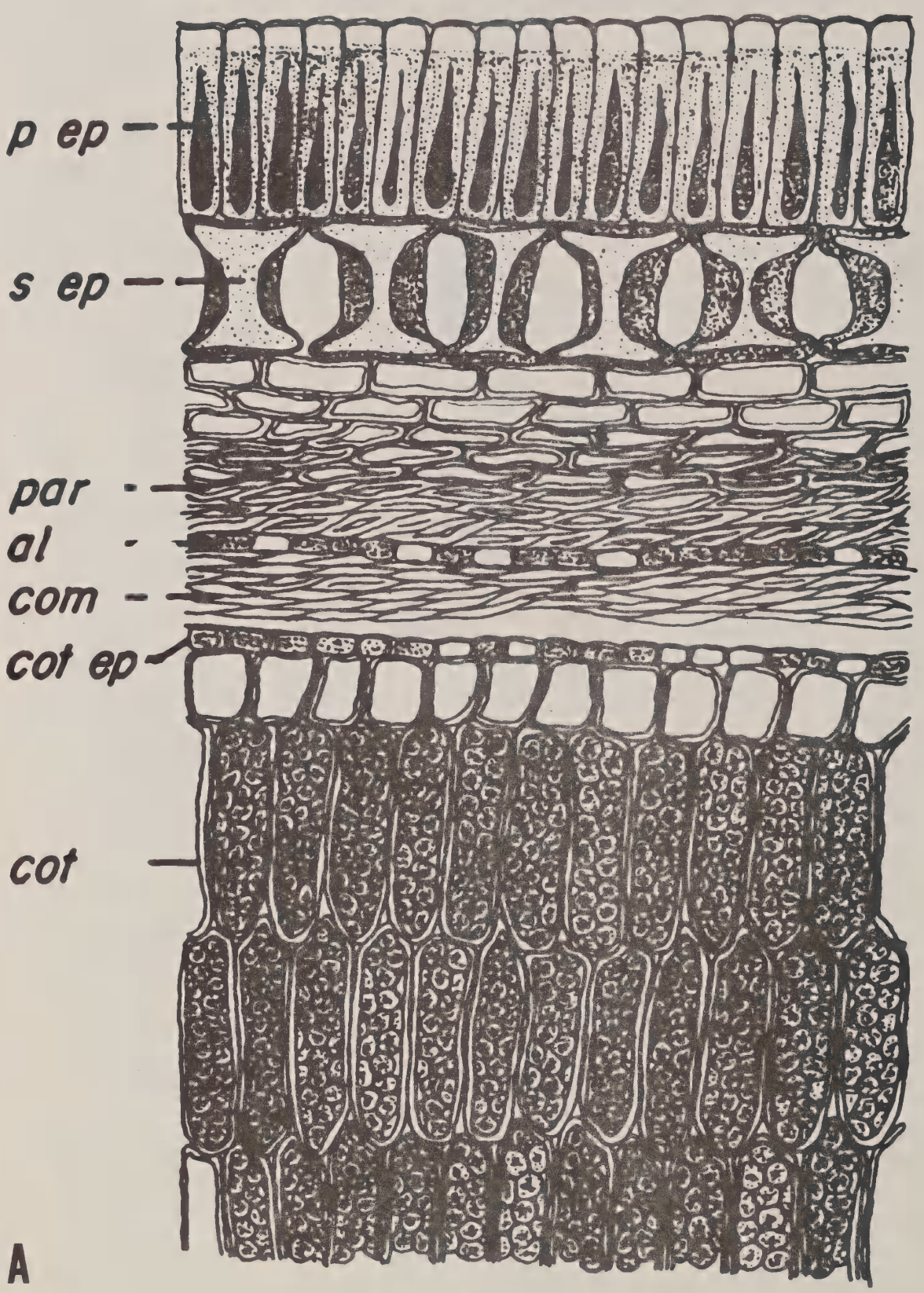
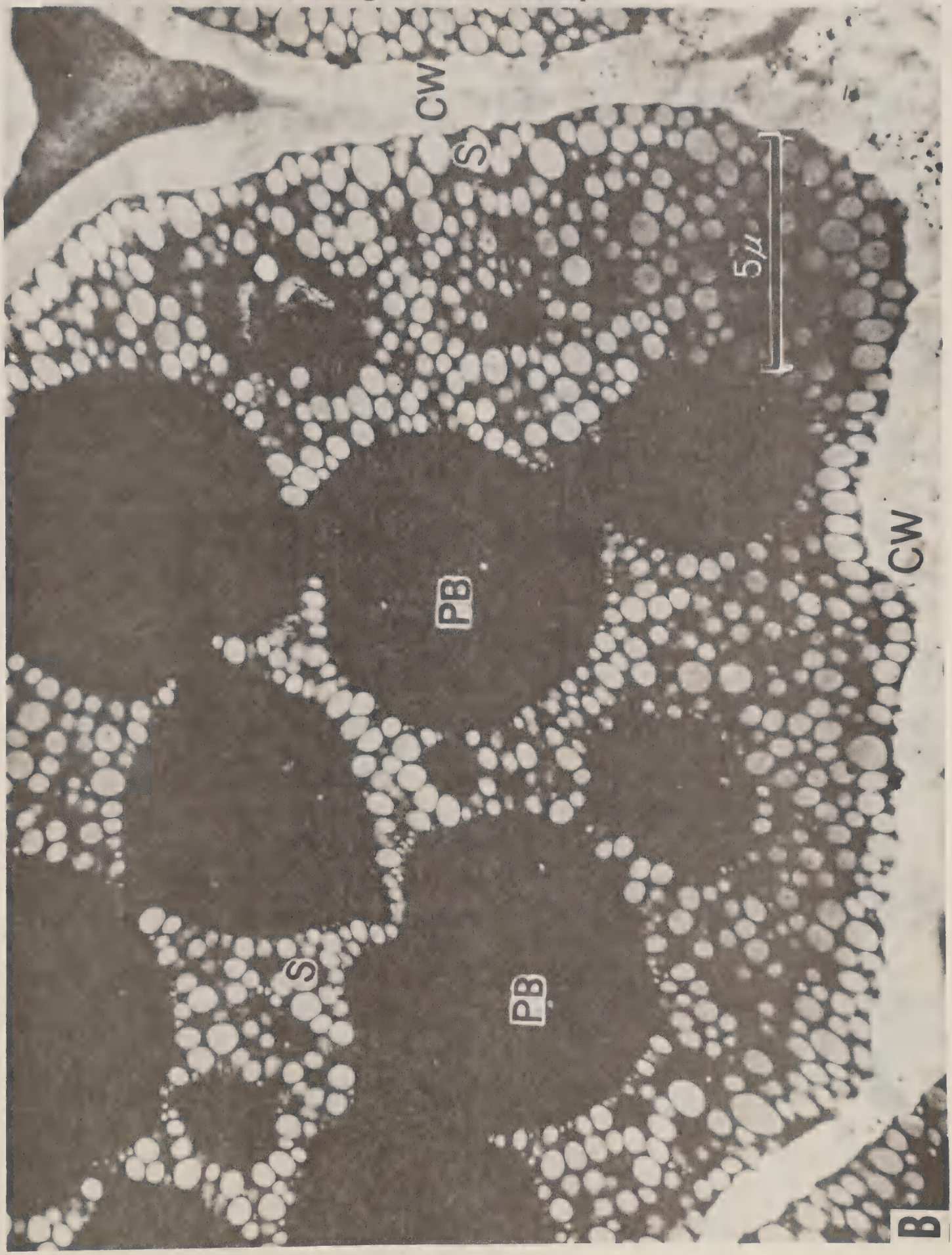
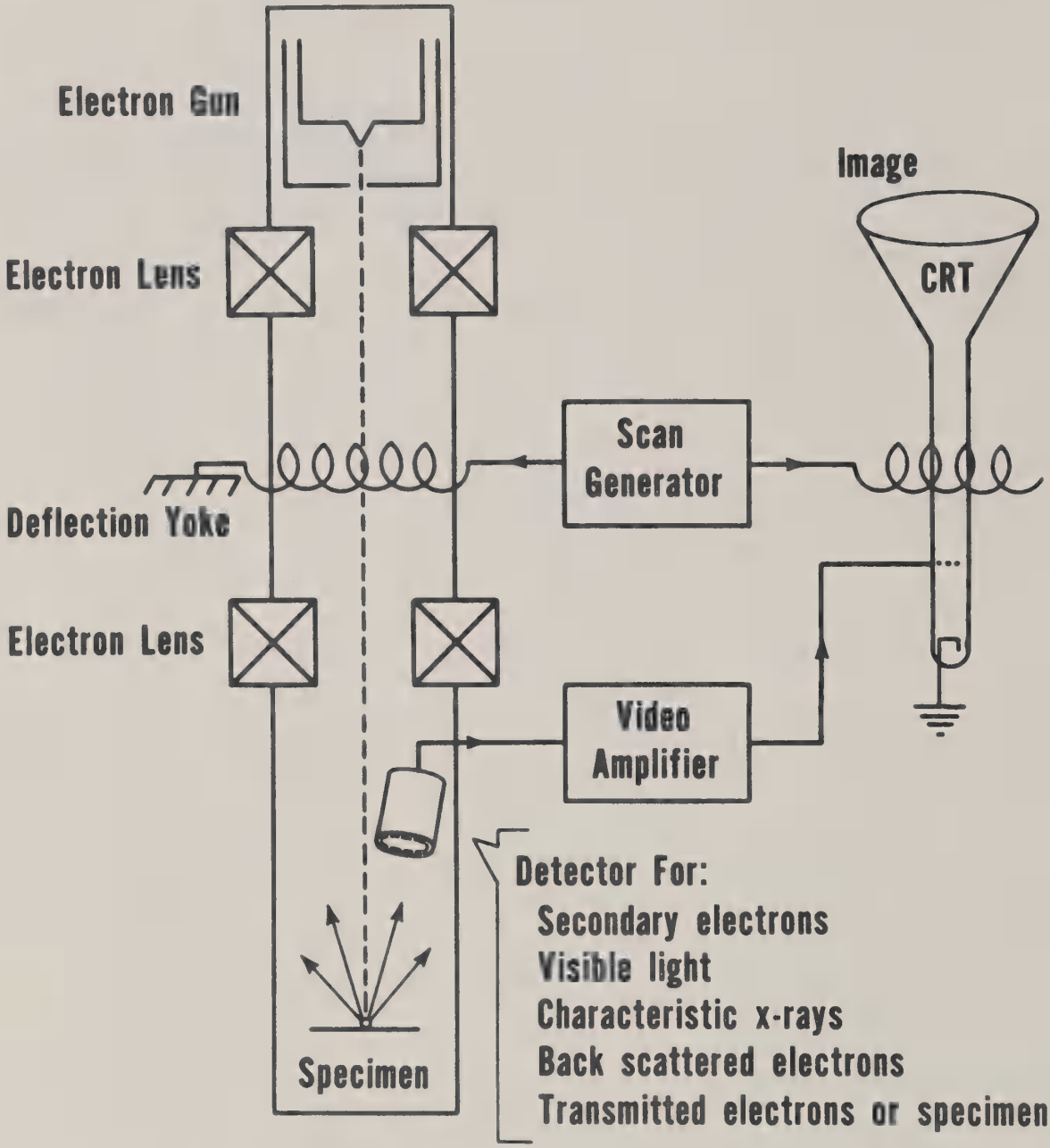


Figure 10


p ep
s ep
par
al
com
cot ep
cot
A


CW
S
5μ
PB
CW
S
PB
B



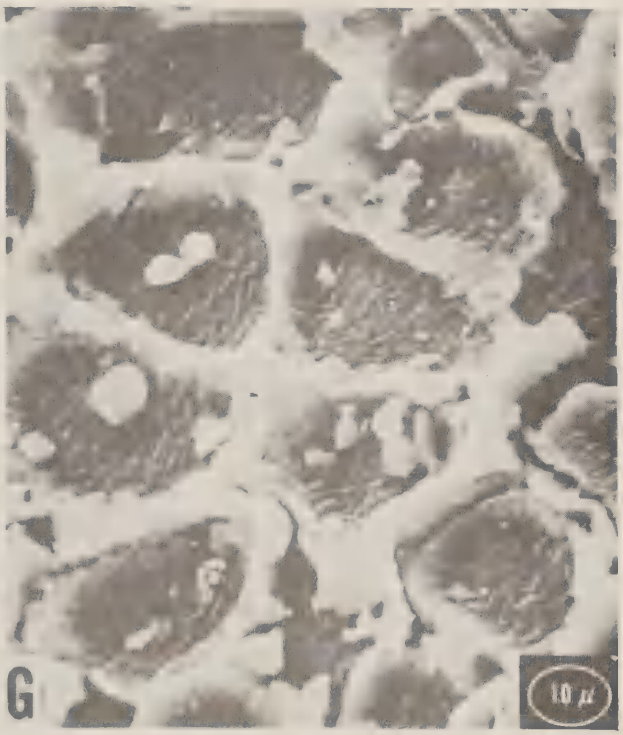
T.L. Hayes & R.F.W. Pease, Ann. N.Y. Acad. Sci., 157, 497(1969)

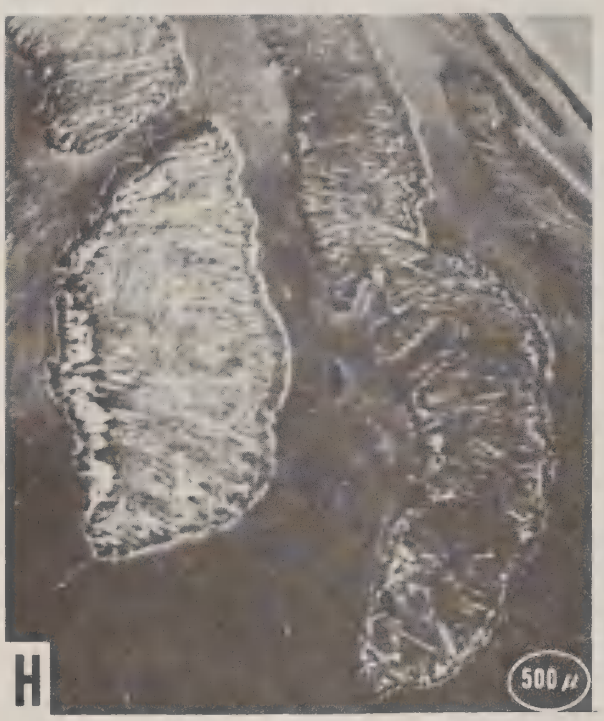
C


D
200 μ

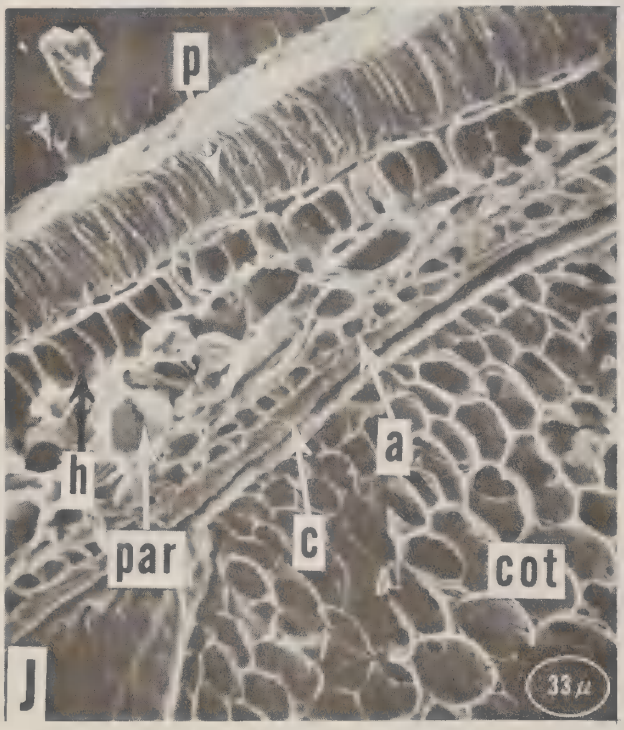

E
5 μ


F
200 μ

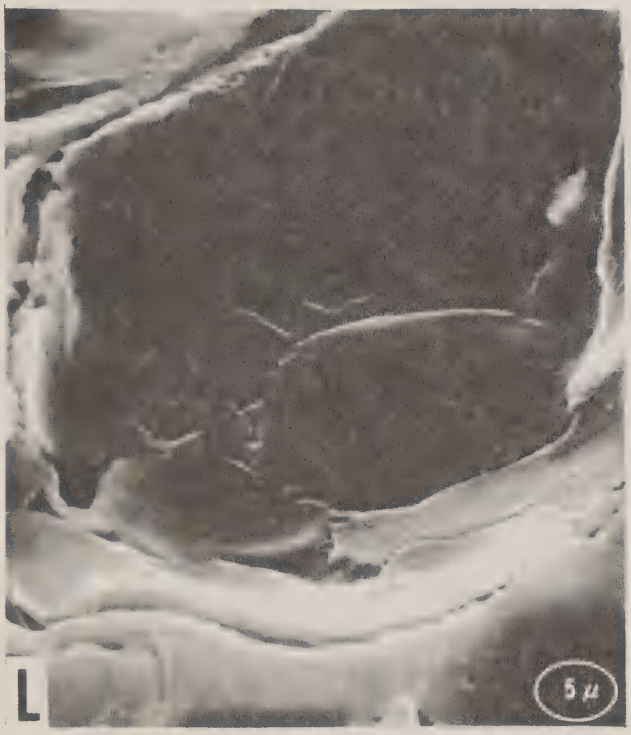

G
10 μ


H
500 μ


I
50 μ

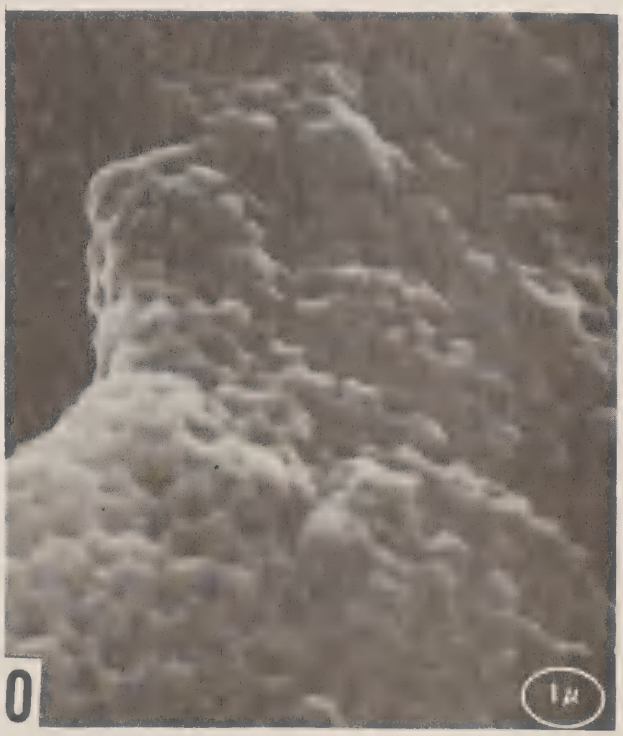

p
h
par
c
a
cot
J
33 μ


K
10 μ


L
5 μ

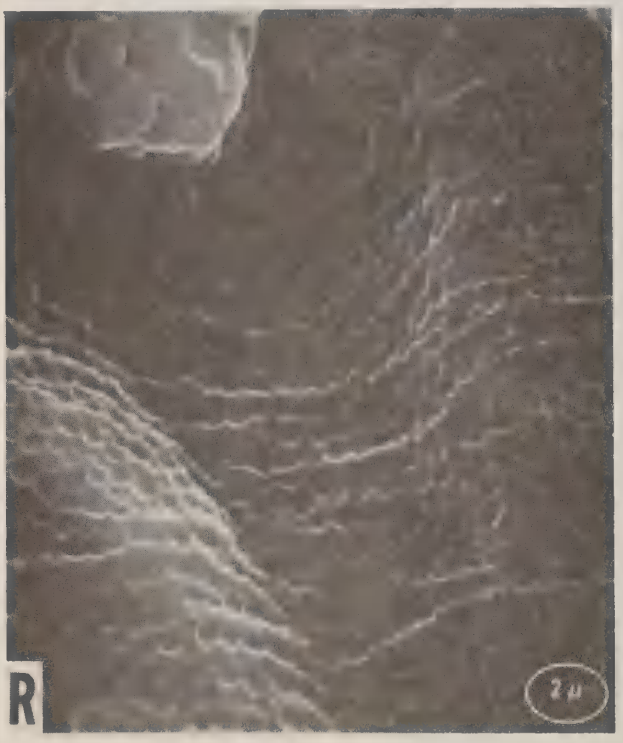

M
20 μ


N
2 μ


O
1 μ


P
20 μ

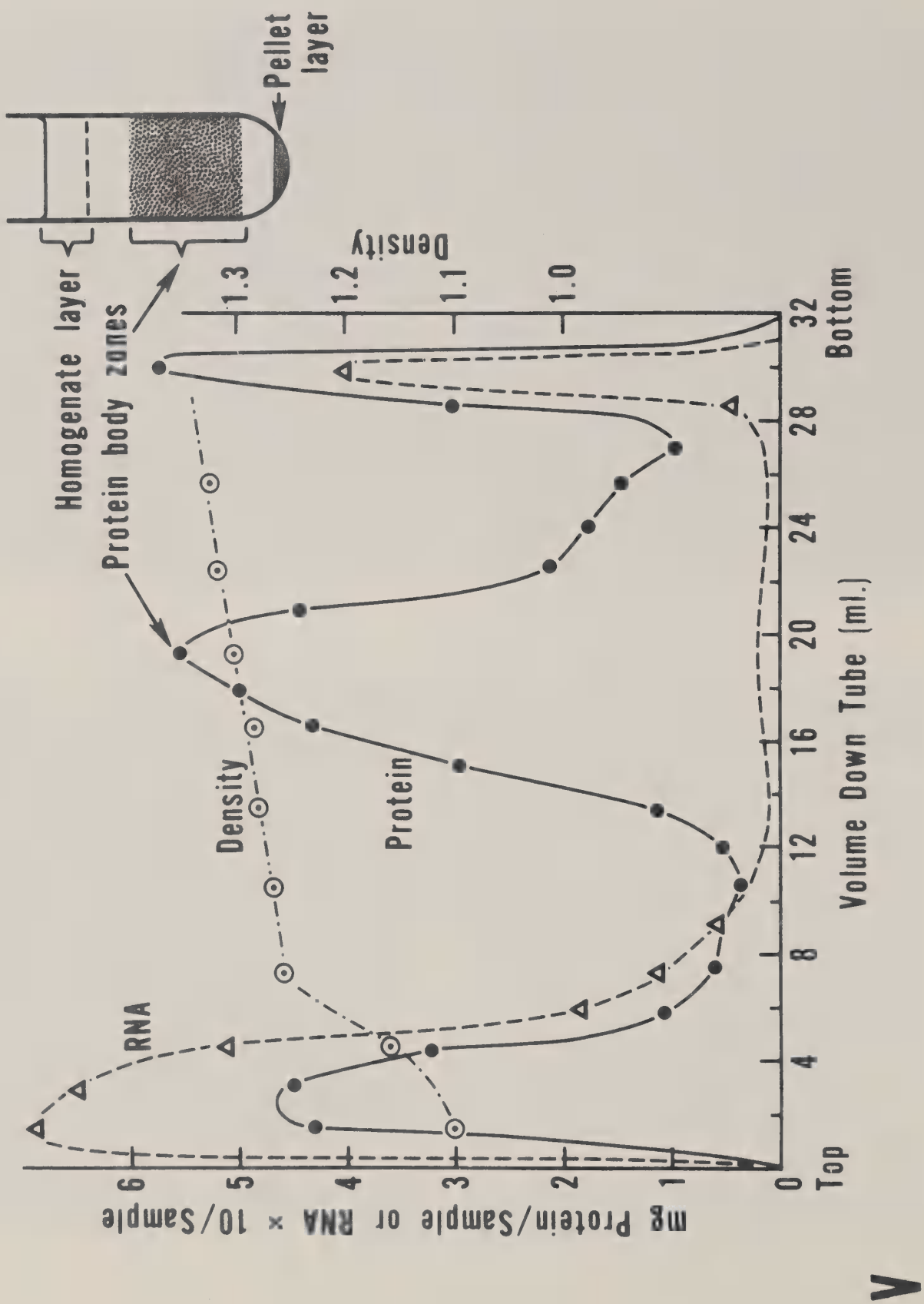

Q
5 μ

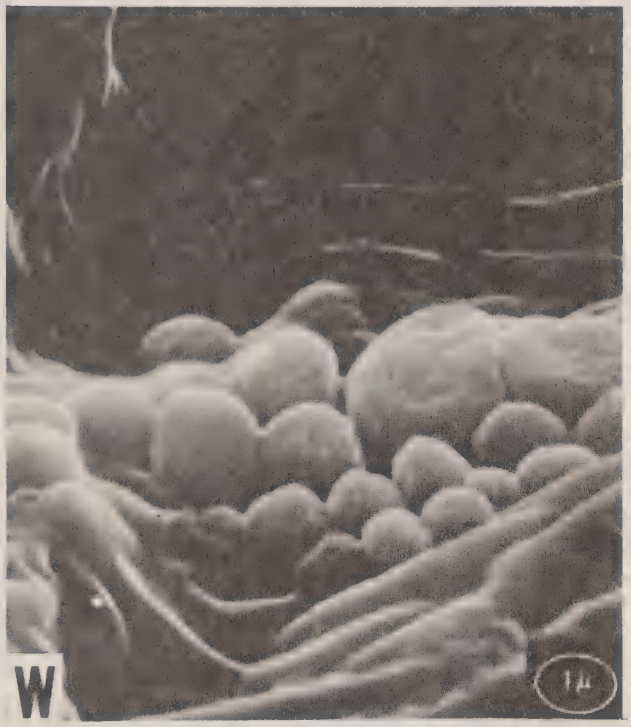

R
2 μ

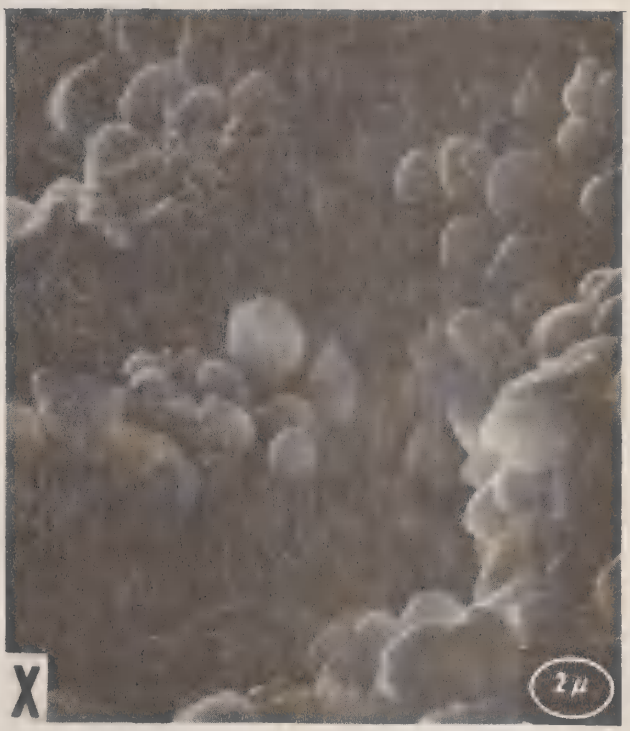

S
20 μ


T
3μ

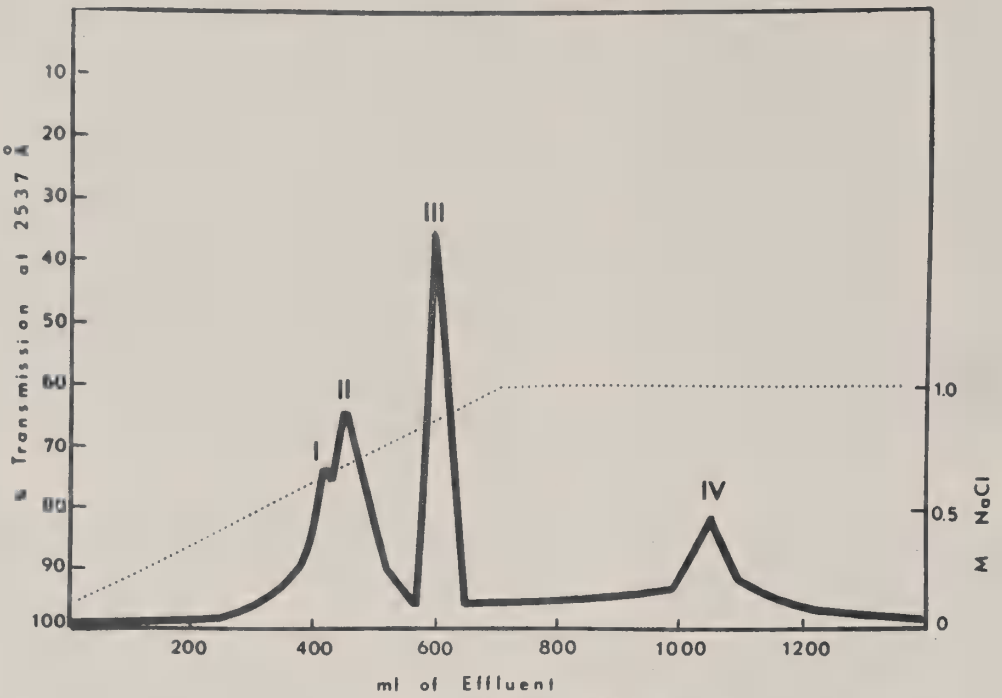

U
1μ


Pellet layer
Homogenate layer
Protein body zones
Density
1.3
1.2
1.1
1.0
Bottom
32
28
24
20
16
12
8
4
Top
Volume Down Tube (ml.)
Density
Protein
RNA
mg Protein/Sample or RNA × 10/Sample
0
1
2
3
4
5
6

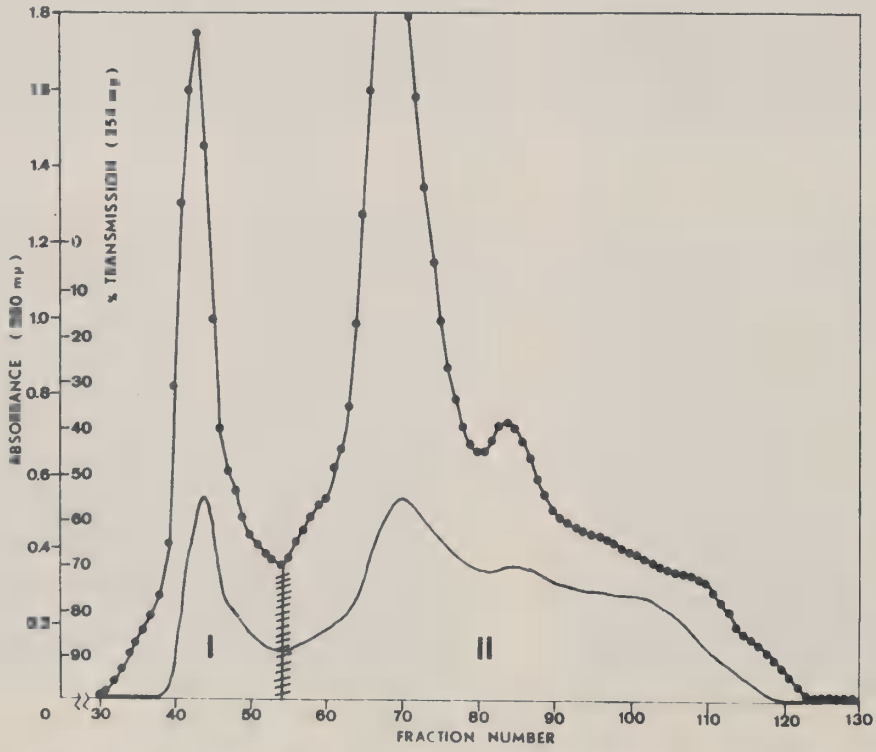

W
1μ


X
2μ

Protein Bodies
Homogenate Fraction
Buffer Extract
2S 7S 11S 15S
2S 7S 9S 11S
0.5μ
0.1μ
Y



Chromatography of fraction X with DEAE-Sephadex A-50. Column size: 4 × 40-cm. Sample: 200 mg. of fraction X in 10 ml. of pH 7.6 phosphate buffer containing 0.1*M* NaCl and 0.01*M* mercaptoethanol. Elution: same buffer containing NaCl in gradient concentration of 0 to 1.0*M*, flow rate 20 ml./hr. Full line: UV absorption at 2537 A, 1-mm. cell. Dashed line: gradient of NaCl concentration.



Elution profile of a crude reserve soybean protein preparation on a 4 × 100-cm Bio-Gel A-1.5 m (100–200 mesh) column. Flow rate: 28 ml/hr. Volume of each fraction: 5.6 ml. Eluant: pH 7.6 phosphate buffer made 0.4 M in NaCl and 0.01 M in mercaptoethanol. Solid line (—): Percent transmission at 254 mμ, 3-mm cell. Solid circles (●—●): Absorbance at 280 mμ, 1-cm cell.

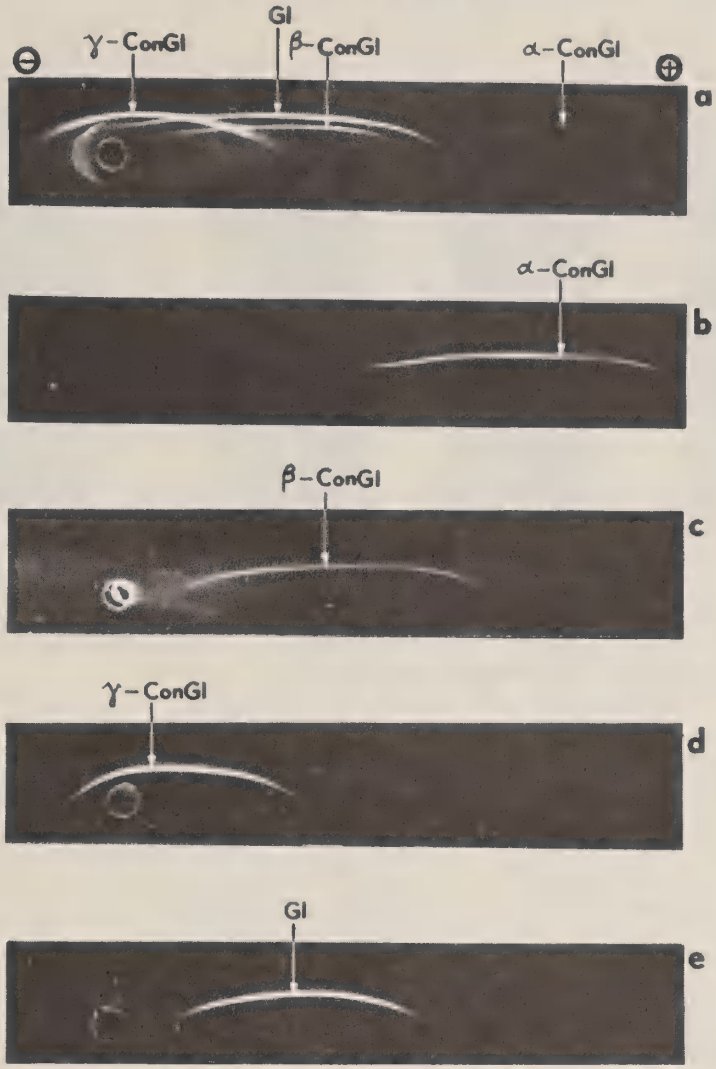


FIG. 2. Immunoelectrophoresis patterns of (a) Four major reserve proteins of soybean seeds, (b) α-conglycinin, (c) β-conglycinin, (d) γ-conglycinin, and (e) glycinin. An anti-soybean water extract serum 123 was used for the development of the immunoprecipitin bands.

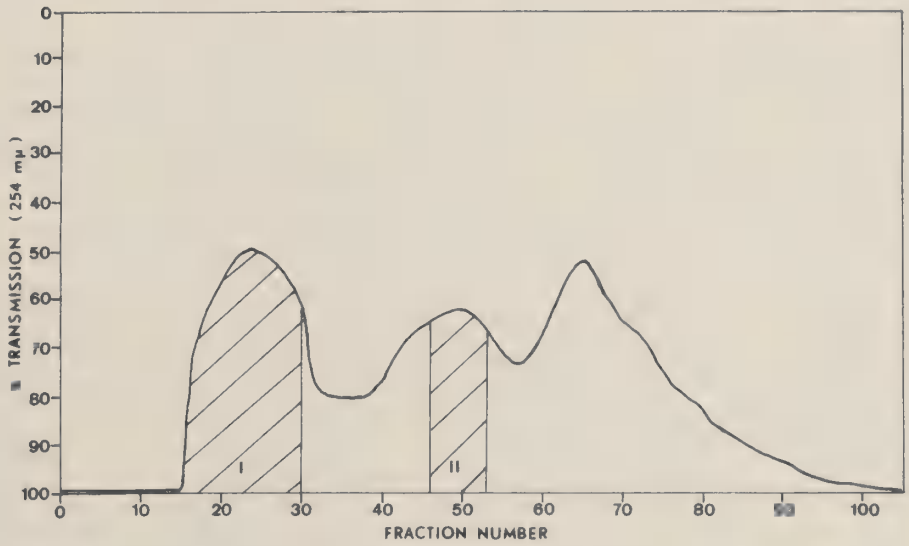
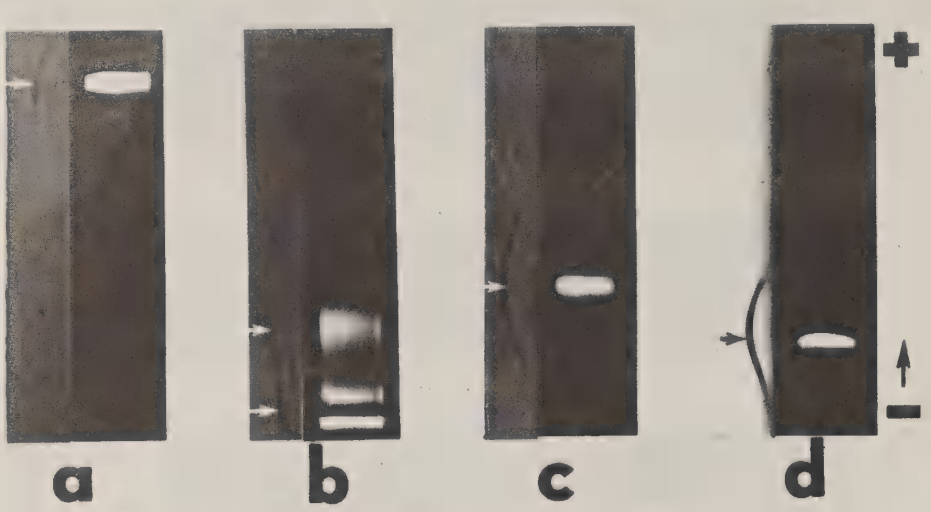
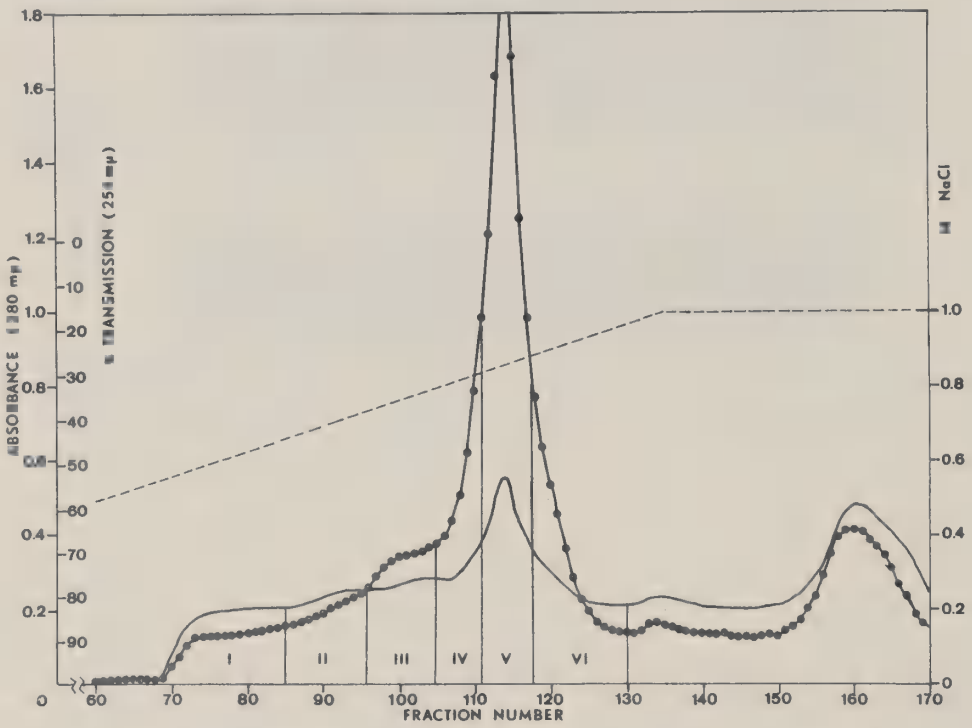


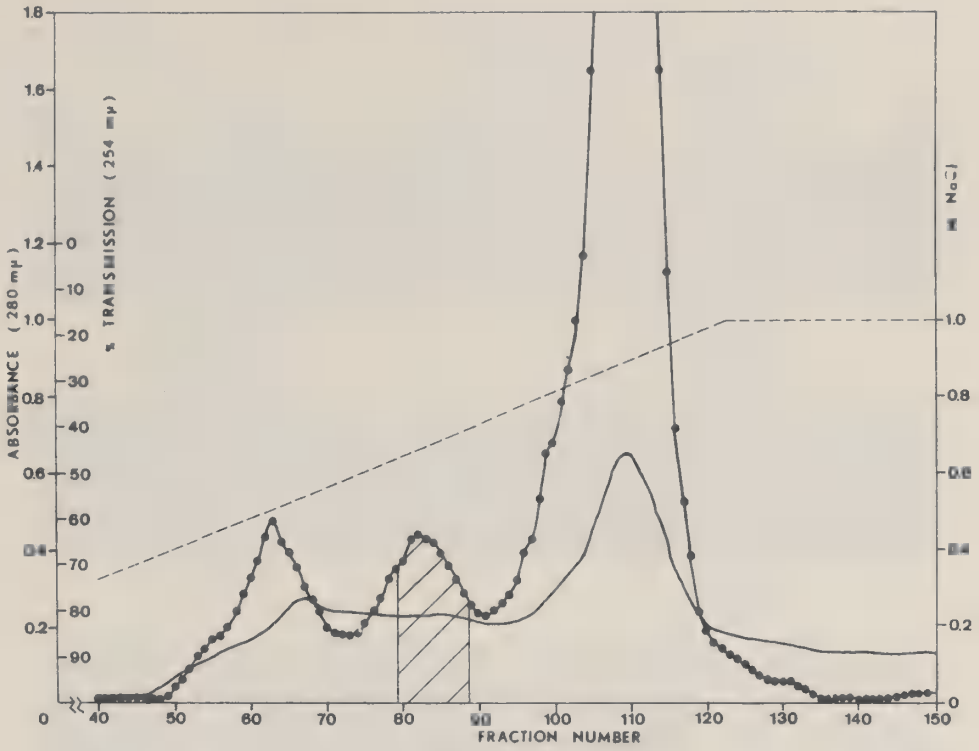
FIG. 3. Elution profile of a crude 7S soybean protein preparation (6) on a 4 × 100-cm Sephade . G-100 column. Flow rate: 28 ml/hr. Volume of each fraction: 8.0 ml. Eluant: pH 7.6 phosphate buffer made 0.4 M in NaCl and 0.01 M in mercaptoethanol. Solid line (——): Percent transmission at 254 mμ, 3-mm cell.



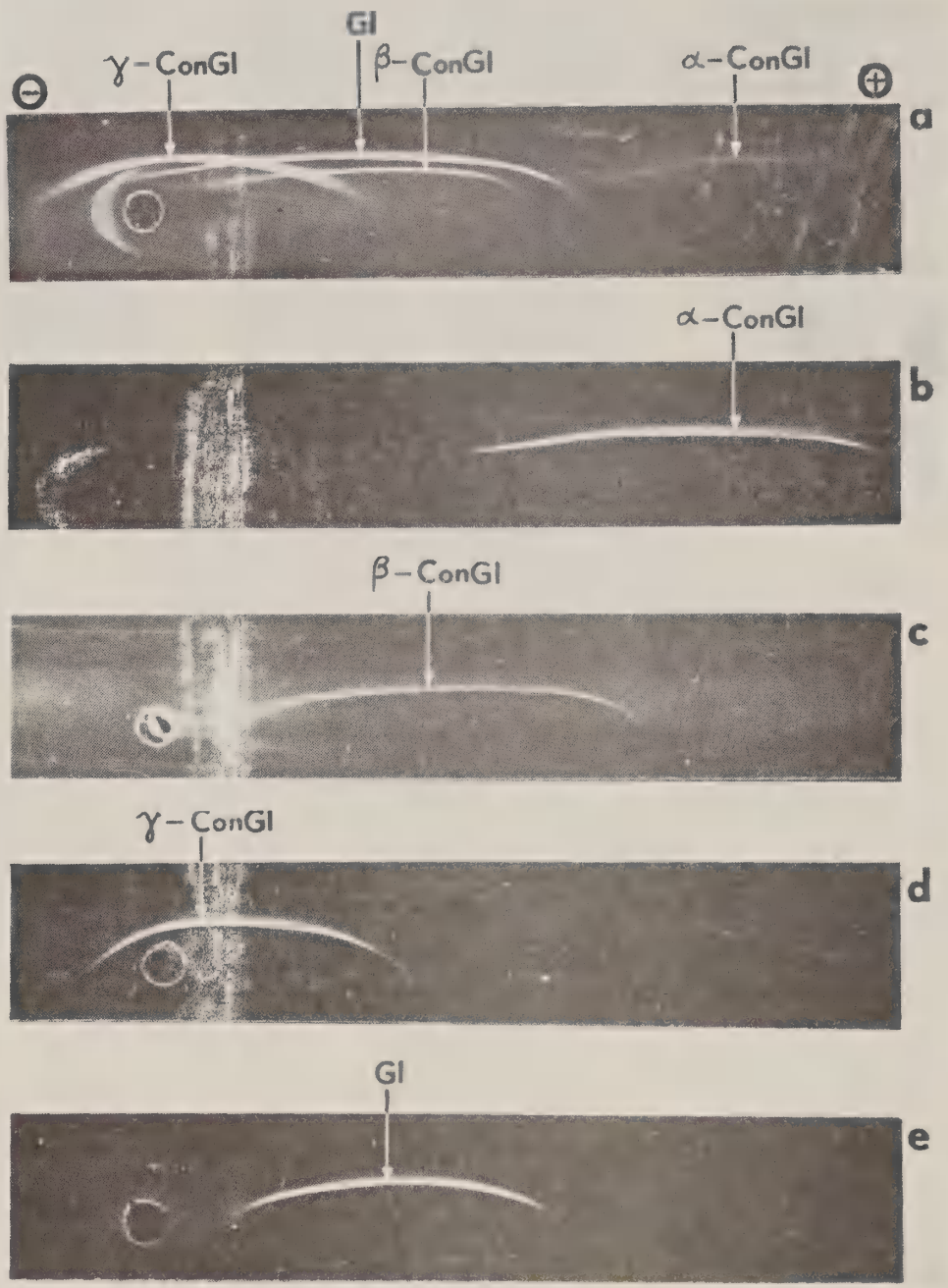
Disc electrophoresis and disc immuno-electrophoresis patterns of: (a) α-conglycinin, (b) β-conglycinin, (c) γ-conglycinin, and (d) glycinin. An anti-soybean water extract serum 123 was used for the development of the immunoprecipitin bands.



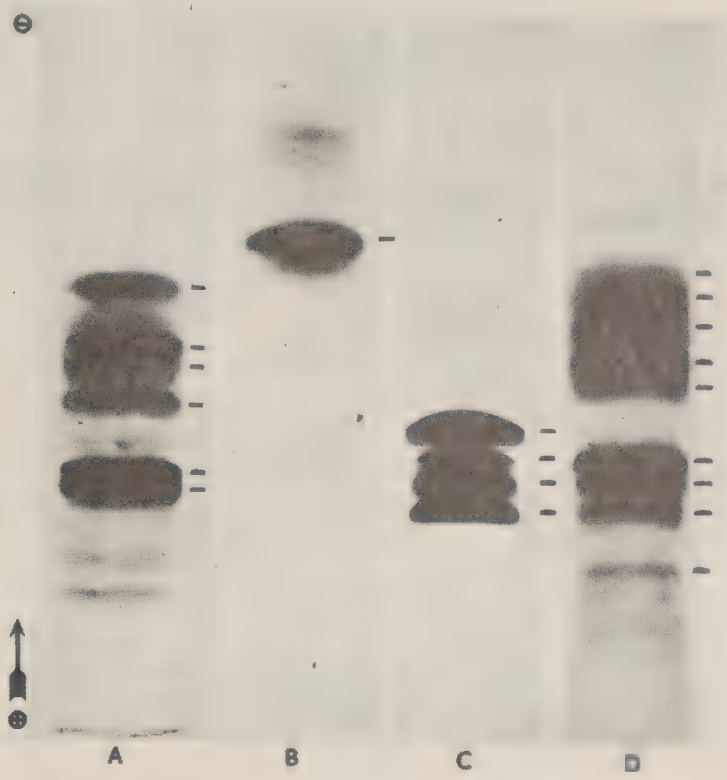
Chromatography of a crude 7S soybean protein preparation (7) on a 4 × 40-cm DEAE-Sephadex A-50 column equilibrated with pH 7.6 phosphate buffer made 0.1 M in NaCl an 0.01 M in mercaptoethanol. Elution: Same buffer containing NaCl in gradient concentration of 0.1 to 1.0 M. A linear gradient system was started by using two 500 ml chambers in hydrostatic equilibrium. The mixer chamber contained 350 ml of the pH 7.6 buffer and the reservoir chamber an equal volume of 1.0 M NaCl adjusted to pH 7.6. When the eluate was 1.0 M in respect to NaCl, elution was continued by placing 1.0 M of NaCl adjusted to pH 7.6 in both chambers. Flow rate: 24 ml/hr. Volume of each fraction: 5 ml. Solid line (——): Percent transmission at 254 mμ, 3-mm cell. Solid circles (●): Absorbance at 280 mμ, 1-cm cell. Dashed line (– – –): Gradient of NaCl concentration.



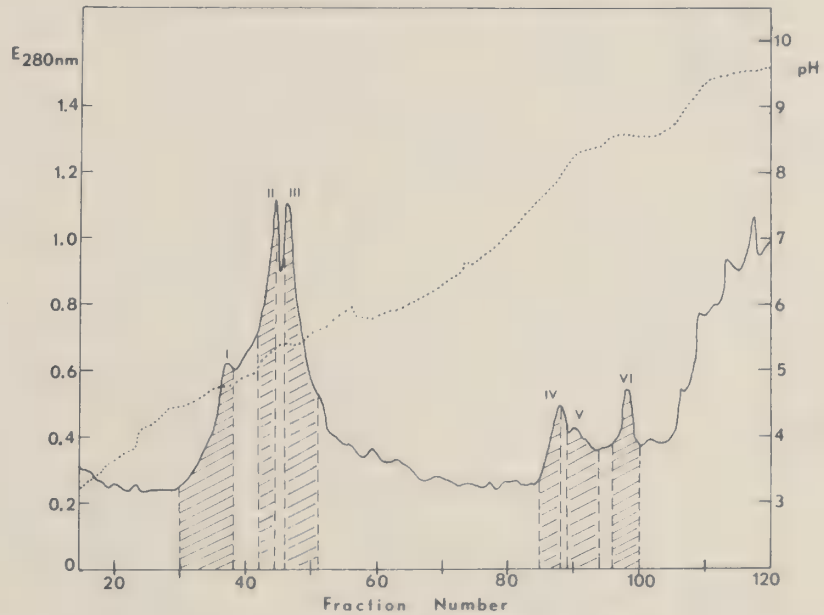
Chromatography of Bio-Gel A-1.5m Fraction II preparation on a 4 × 40-cm DEAE-Sephadex A-50 column equilibrated with pH 7.6 phosphate buffer made 0.1 M in NaCl and 0.01 M in mercaptoethanol. Elution: Same buffer containing NaCl in gradient concentration of 0.1 to 1.0 M. A linear gradient system was started by using two 500 ml chambers in hydrostatic equilibrium. The mixer chamber contained 350 ml of the pH 7.6 buffer and the reservoir chamber an equal volume of 1.0 M NaCl adjusted to pH 7.6. When the eluate was 1.0 M in respect to NaCl, elution was continued by placing 1.0 M of NaCl adjusted to pH 7.6 in both chambers. Flow rate: 24 ml/hr. Volume of each fraction: 5 ml. Solid line (——): Percent transmission at 254 mμ, 3-mm cell. Solid circles (●): Absorbance at 280 mμ, 1-cm cell. Dashed line (– – –): Gradient of NaCl concentration.



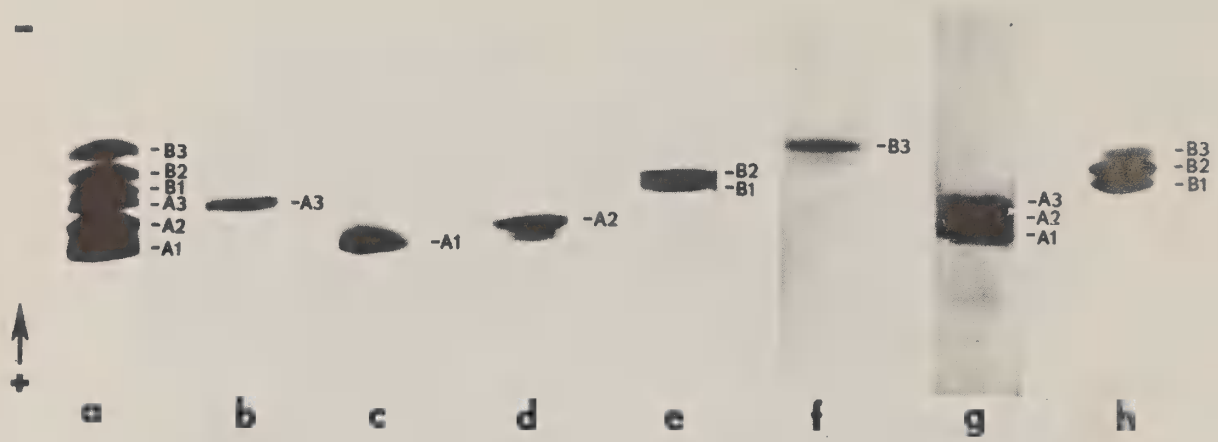
Immunoelectrophoresis in agar gel patterns of (a) four major reserve proteins of soybean seeds, (b) alpha-conglycinin, (c) beta-conglycinin, (d) gamma-conglycinin, and (e) glycinin. A polyvalent pooled antisoybean water extract serum was used for the development of the immunoprecipitin bands. Immunoelectrophoresis in agar gel was carried out by the general procedure described in Grabar and Williams (10) as modified by Scheidegger (11). The gel medium consists of 1% Ionagar No. 2 (Oxoid) in pH 8.8 buffer, tris-barbital-sodium barbital, 0.05 ionic strength (Gelman). Electrophoresis was carried out for 2.5 hr. with a current of 5 ma. per microscope slide. Staining of the precipitin arcs was done with Ponceau S as described by Uriel (12).



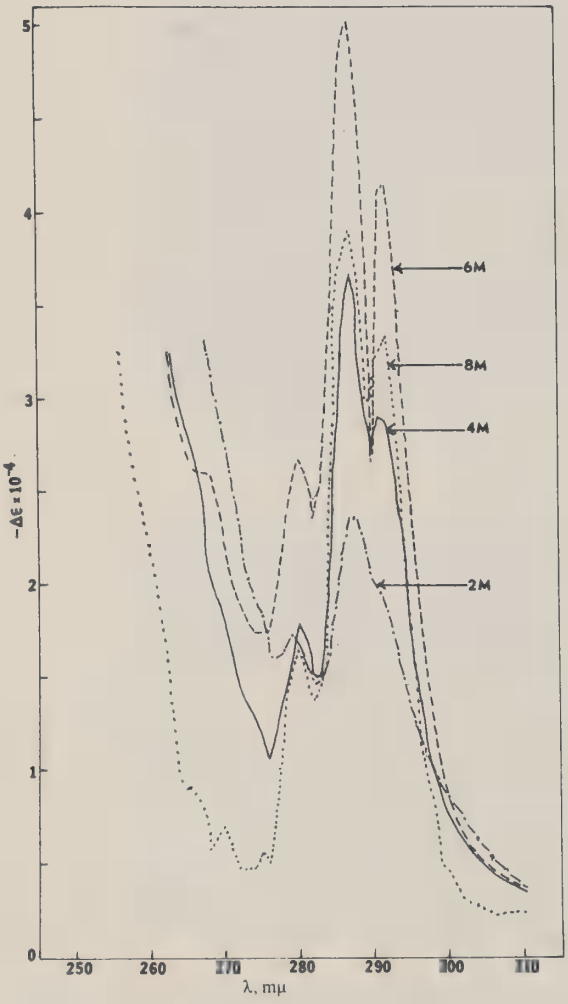
Disc electrophoresis of the major reserve proteins of soybean seeds after dissociation in the solvent system phenol-acetic acid-0.2M mercaptoethanol (2:1:1, w./v./v.) made 8M in urea. The polyacrylamide gels were equilibrated with the same solvent (ref. 5). Key: A, glycinin; B, alpha-conglycinin; C, beta-conglycinin; and D, gamma-conglycinin.



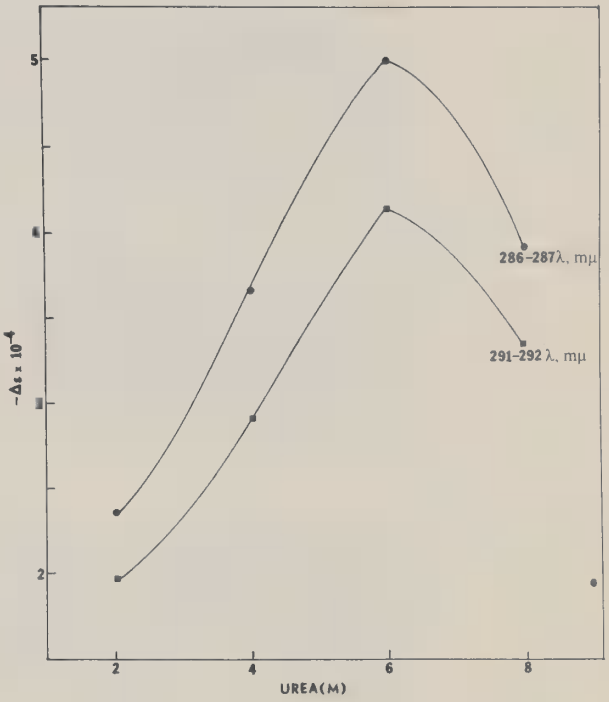
Isoelectric focusing of dissociated glycinin (100 mg) in the region between pH 3 and pH 10 in the presence of 6 M urea and 0.2 M mercaptoethanol. The solid line represents absorbance at 280 nm (1 cm cell). The dotted line shows the pH gradient measured at 25°. Shaded areas indicate fractions pooled (I through VI).



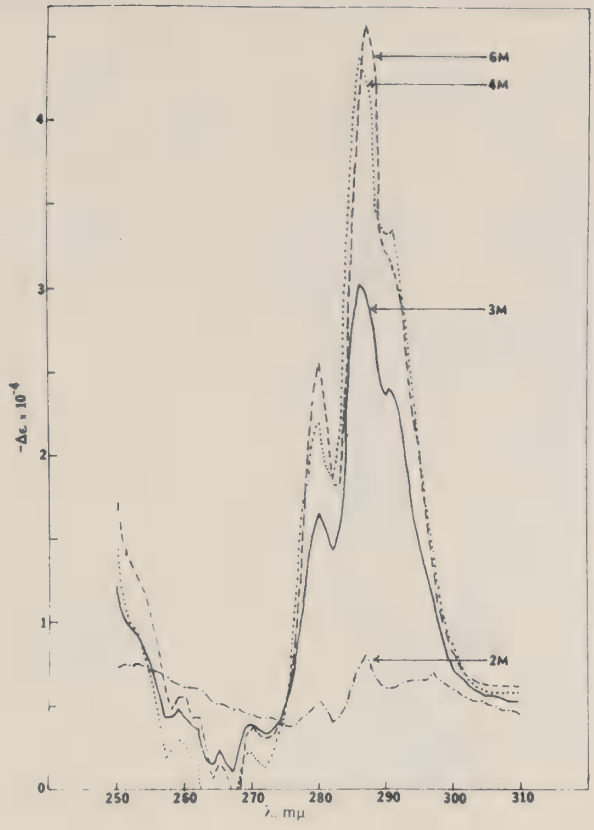
Disc electrophoresis in PAMU solvent of dissociated glycinin (a), fraction I (b), fraction II (c), fraction III (d), fractions IV or V (e), fraction VI (f), pooled fractions I–III (g), and pooled fractions IV–VI (h). Acidic subunits have been designated "A", and basic subunits "B".



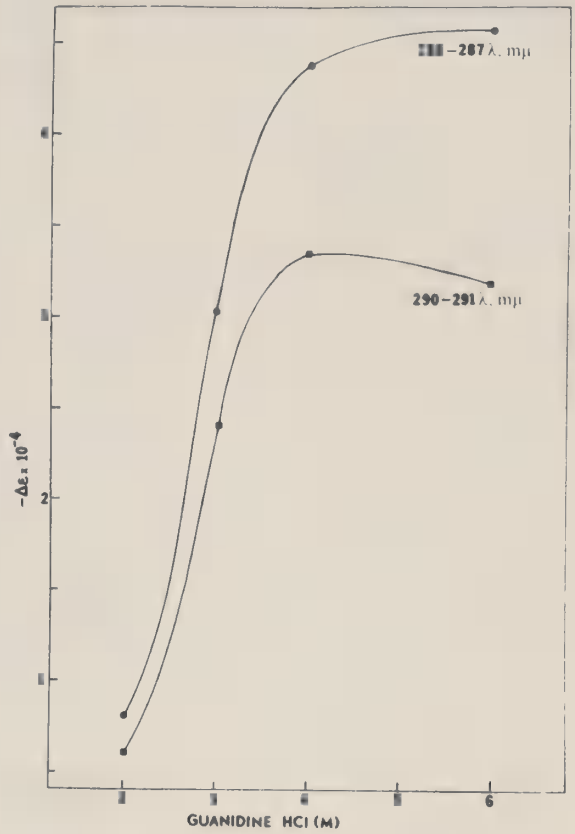
Ultraviolet difference spectra of glycinin in different concentrations of urea.



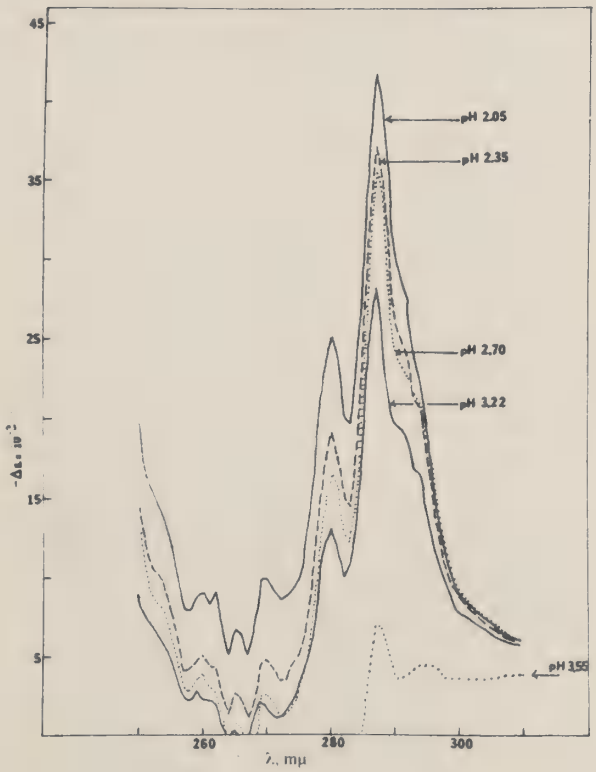
Variation of the molar absorptivity difference ($\Delta\varepsilon$) of glycinin at 286–287 mμ and 291–293 mμ as a function of urea concentration.



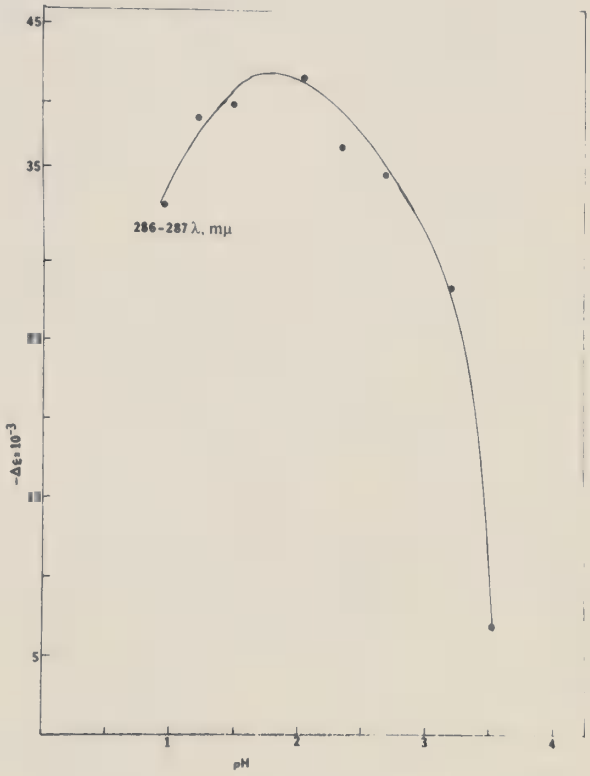
Ultraviolet difference spectra of glycinin in different concentrations of guanidine hydrochloride.



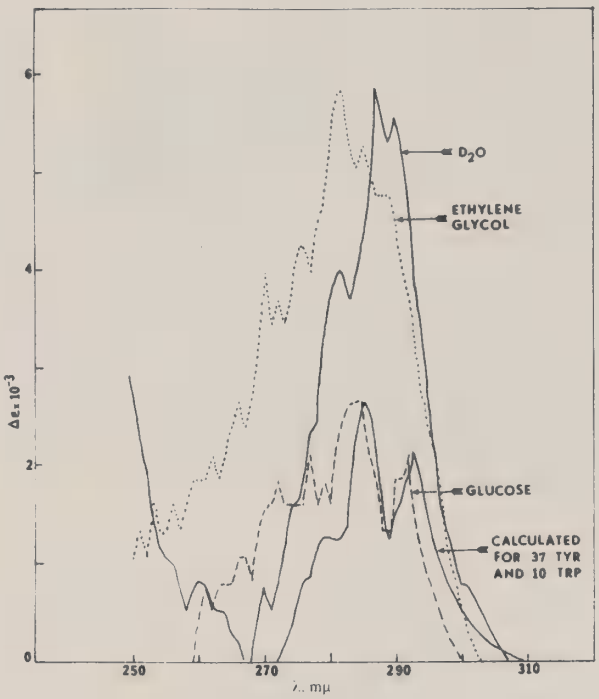
Variation of the molar absorptivity difference ($\Delta\varepsilon$) of glycinin at 286–287 mμ and 290–291 mμ as a function of guanidine hydrochloride concentration.



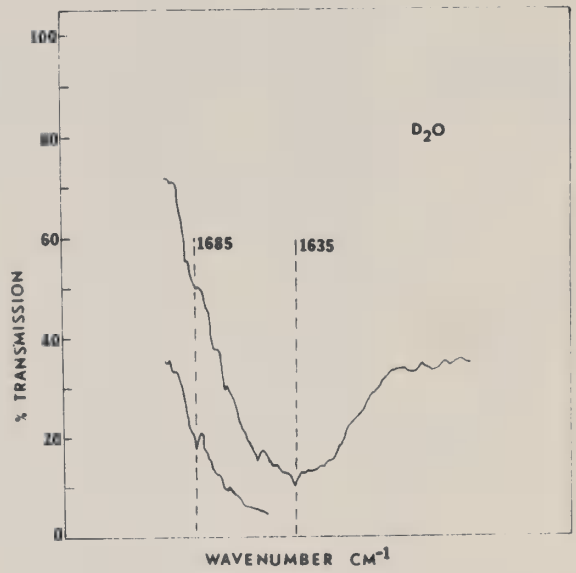
Acid-induced ultraviolet difference spectra of glycinin.



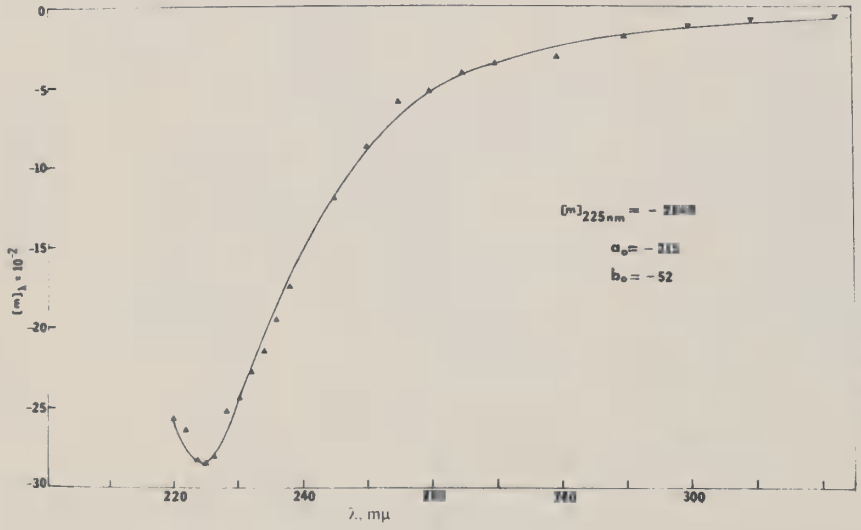
Variation of the molar absorptivity difference ($\Delta\varepsilon$) of glycinin at 286–287 mμ as function of pH.



Solvent-induced perturbation spectra (ultraviolet) of native glycinin in the presence of 90% D_2O, 20% ethylene glycol, and 20% glucose. The molar absorptivity difference ($\Delta\varepsilon$) values are positive in the presence of ethylene glycol and glucose but negative in the presence of D_2O. The values are shown in this diagram without positive or negative notation.



Infrared spectra of native glycinin in D_2O around the amide I region.



The optical rotatory dispersion curve of native glycinin.

Amino acid composition of glycinin

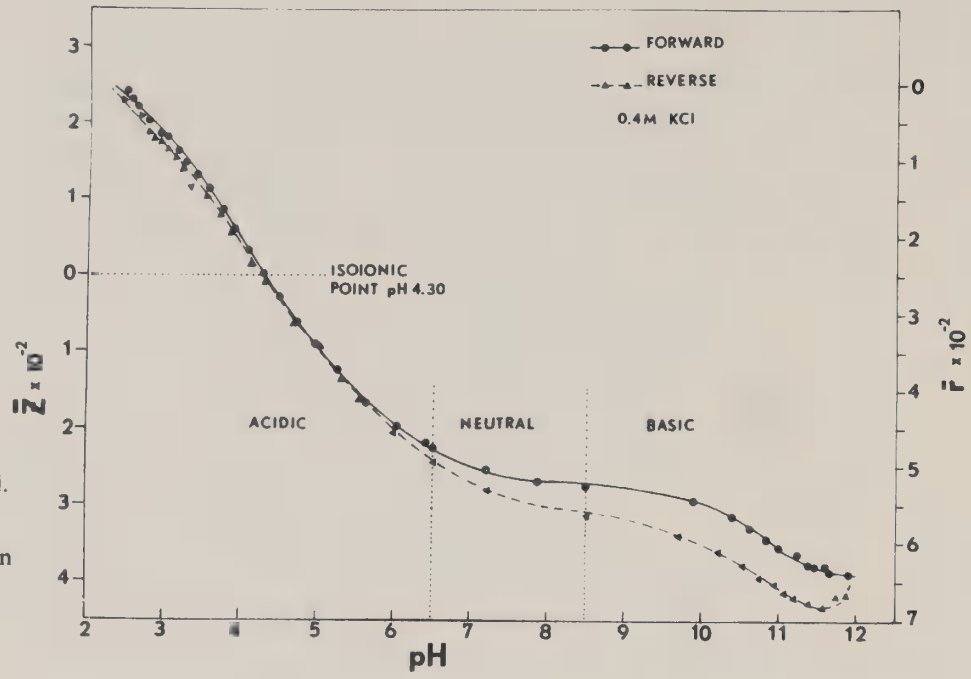
Amino acid	g residue/ 100 g protein[a]	No. residues/ 350,000 g[b]
Lysine	4.97 ± 0.02	136 ± 0
Histidine	2.26 ± 0.12	58 ± 4
Arginine	7.98 ± 0.01	178 ± 0
Aspartic acid	12.01 ± 0.12	364 ± 4
Threonine	3.45 ± 0.05	120 ± 2
Serine	5.35 ± 0.06	204 ± 2
Glutamic acid	22.06 ± 0.05	612 ± 2
Proline	5.83 ± 0.13	210 ± 6
Glycine	3.76 ± 0.06	230 ± 4
Alanine	3.16 ± 0.01	158 ± 6
Cystine $^1/_2$	1.40[c] ± 0.20	44 ± 6
Valine	4.18 ± 0.09	148 ± 4
Methionine	1.16 ± 0.05	30 ± 2
Isoleucine	4.22 ± 0.07	130 ± 2
Leucine	6.97 ± 0.01	216 ± 0
Tyrosine	4.05 ± 0.06	86 ± 2
Phenylalanine	4.95 ± 0.07	118 ± 2
Tryptophan		23[d]

[a] Average of four determinations and standard deviation.
[b] To the nearest even integral numbers (350,000 M.W. = dimer).
[c] Determined as cysteic acid.
[d] Determined spectrophotometrically (12).

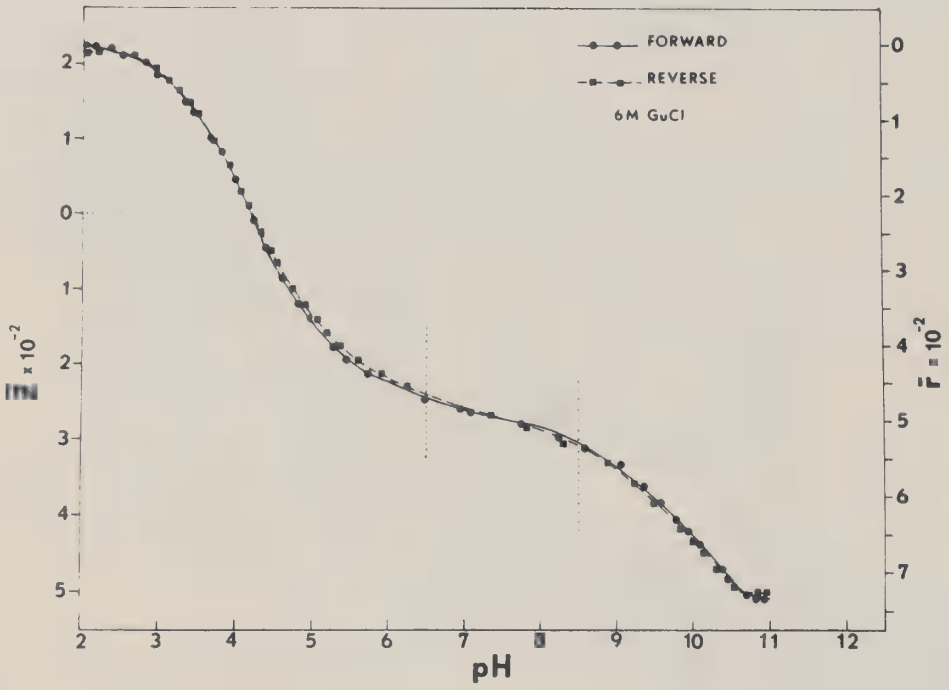
Ionizable amino acid side-chains in glycinin

Ionizable group	Forward titr. in 0.4M KCl	Backward titr. in 0.4M KCl	Fwd.-Bwd. titr. in 6M GuCl	Determined by amino acid anal.
Total acidic	475[a](104)[n]	490[a](108)	475[a](104)	455[b](976[m] ± 6)
Total neutral	50[c] (71)	70[c](100)	60[c] (86)	70[d] ± 4
Total basic	115[e] (52)	155[e] (70)	200[e] (90)	222[f] ± 2
Total ionizable	640 (86)	715 (96)	735 (98)	747[l] ± 6
γ-Carboxyl[g]	463 (105)	475 (107)	460 (104)	443
Phenoxy (tyrosine)	61[h]	61[h] (71)	74[h] (86)	86 ± 2
ε-Amino (lysine)	54[i]	94[i] (69)	126[i] (93)	136 ± 0
Imidazole (histidine)	38[j] (66)	58[k](100)	48[j] (83)	58 ± 4

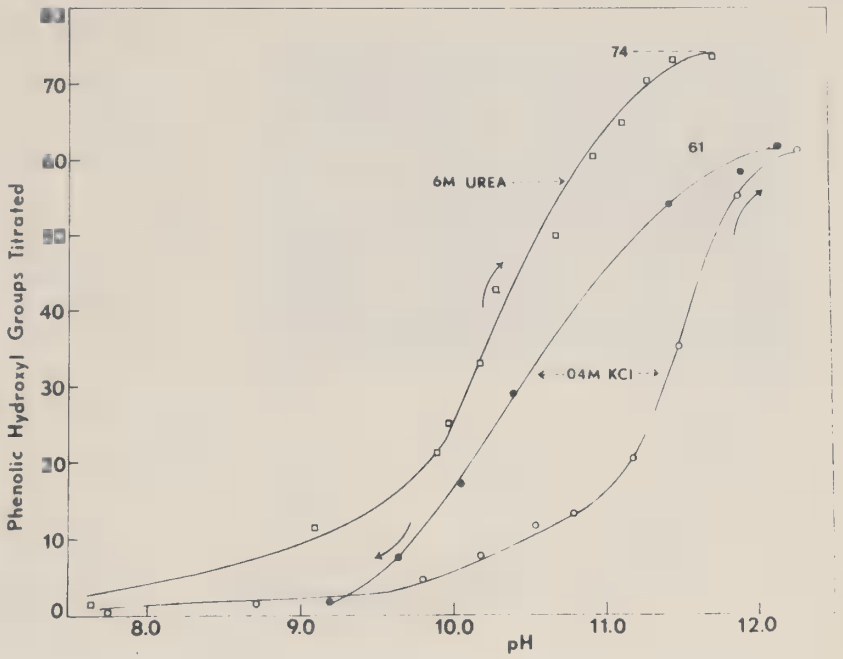
[a] Titrated in the pH 2.0 to 6.5 region. Including titration of 12 α-carboxyl groups.
[b] Glutamic plus aspartic acid minus amide including 12 α-carboxyl groups.
[c] Titrated in the pH 6.5 to 8.5 region.
[d] Including counting of 12 α-amino groups.
[e] Titrated in the pH 8.5 to 12.0 region in 0.4M KCl and in the pH 8.5 to 11.0 region in 6M GuCl.
[f] Counting only tyrosine and lysine groups.
[g] Total free acidic minus 12 α-carboxyl.
[h] Determined by spectrophotometric titration in 6M urea which may not coincide with the value in 6M guanidine.HCl.
[i] Counted from total basic groups minus tyrosine groups. The sulfhydryl group contribution (one mole/mole protein) has been omitted. The assumption is made that possible cleavage of SS bonds by alkali does not occur. Arginine has been assumed not to be titrated in this region.
[j] Total neutral groups minus 12 α-amino groups.
[k] Total neutral groups minus 12 α-amino groups.
[l] Arginine was not included in the count.
[m] Including aspartic acid and glutamic acid only.
[n] Numbers in parentheses denote the percentage of groups titrated in relation to the number expected by amino acid analysis.



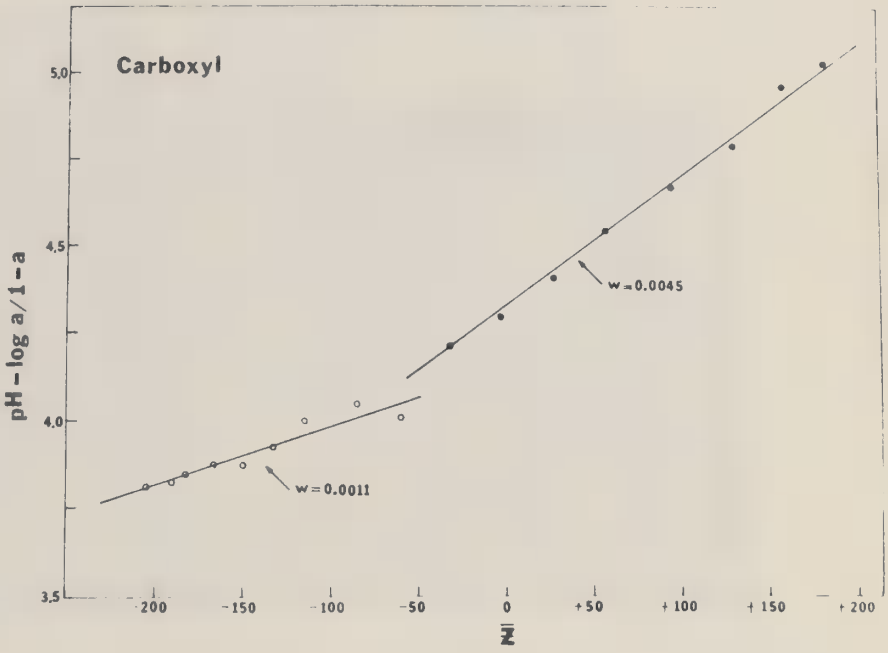
Electrometric titration of glycine in 0.4M KCl. The continuous curve denotes the forward titration and the broken curve the back titration.



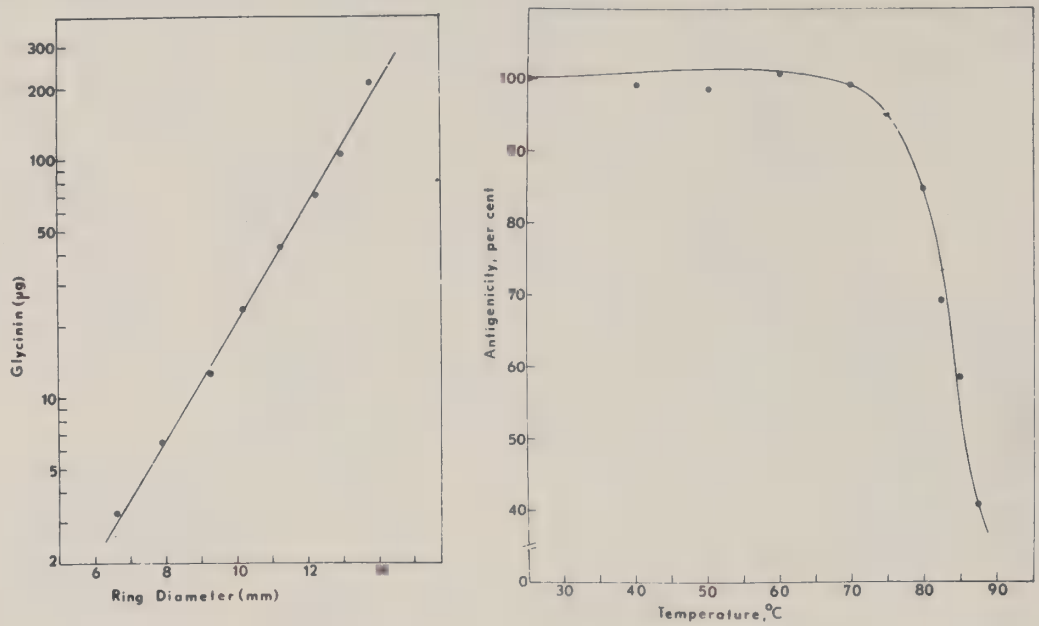
Electrometric titration of glycinin in 6M guanidine hydrochloride. The continuous curve denotes the forward titration and the broken curve the backward titration.



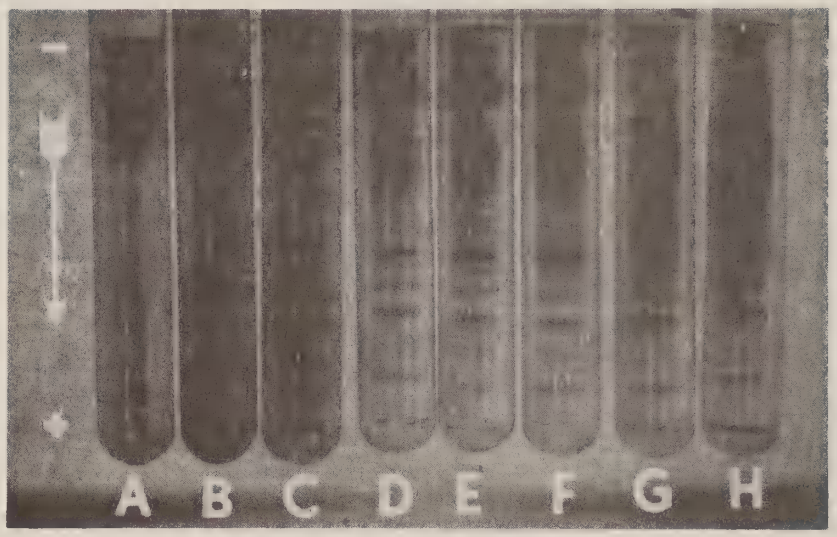
Spectrophotometric titration of tyrosine phenoxy groups of glycinin in 0.4M KCl, forward (o), 0.4M KCl, backward (●), and 6M guanidine hydrochloride, forward (□). Absorbance measurements were made at 295 mμ and converted into the number of phenoxy groups as described in the text.



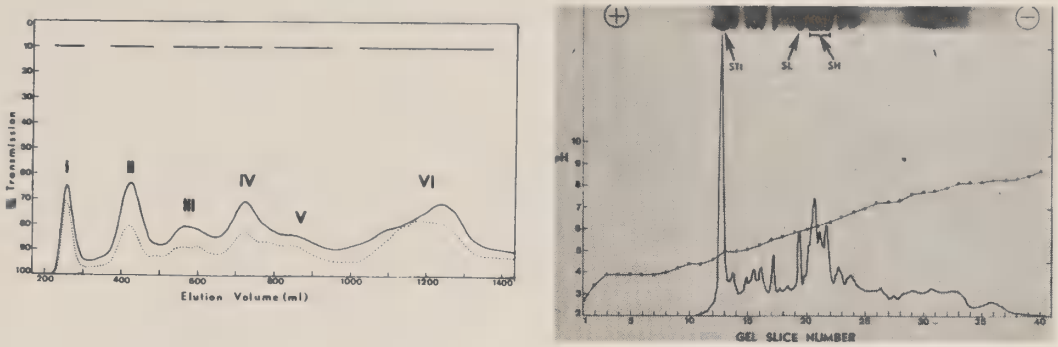
Logarithmic plot of the carboxy region of the titration curves of glycinin in 0.4M KCl. The plot has been divided into two subregions which correspond to pH range and $\bar{Z}$ values explained in the text.



Radial immunodiffusion of glycinin against antiglycinin serum.
The effect of temperature of heating (30 min) on the antigenicity of glycinin.



Disc electrophoresis patterns of glycinin solutions heated for 30 min at 70° (B), 75° (C), 80° (D), 82.5° (E), 85° (F), 87.5° (G), 90° (H), and unheated sample, 25° (A).



Sephadex G-100 elution pattern of the pH 4.5 soluble proteins of soybean cotyledons. ———, percent transmission of the eluates at 280 mμ; — — —, percent transmission at 254 mμ. Solid bars in the upper part of the figure indicate fractions pooled.

Disc electrofocusing on polyacrylamide gel of the pH 4.5 soluble proteins of soybean cotyledons in the pH range between pH 3 and pH 10. The densitometer tracing of the stained electrofocused bands was obtained with a Canalco Model F microdensitometer. Open circles represent the pH gradient along the polyacrylamide gel column after electrofocusing. STI, soybean trypsin inhibitor; SL, soybean lipoxidase; SH, soybean hemagglutinins (glycoproteins).

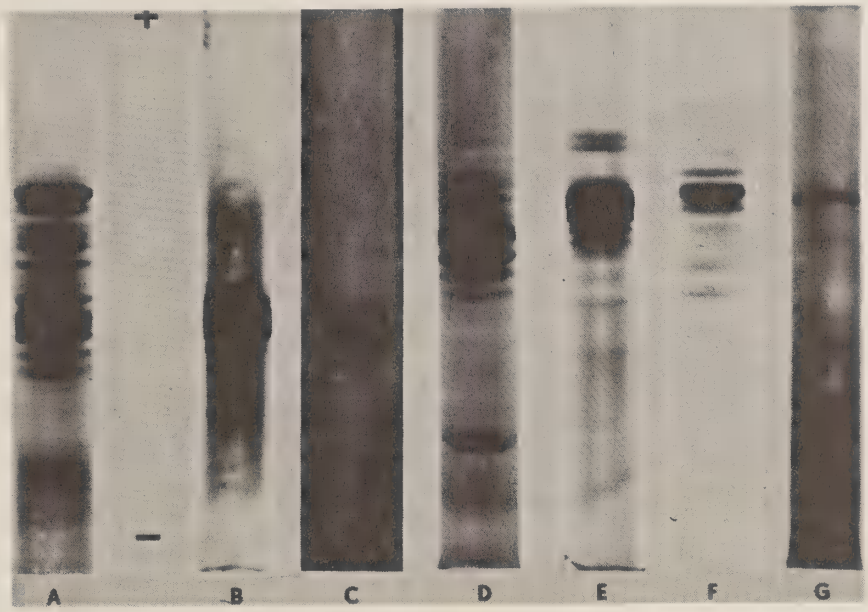
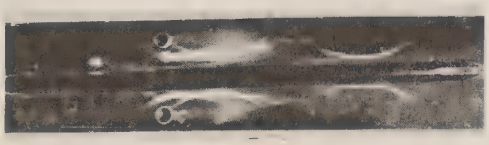


Fig. 3. Disc electrofocusing in the region between pH 3 and pH 10 of: (A) unfractionated pH 4.5 soluble proteins of soybean cotyledons; (B) Fraction II; (C) Fraction II stained for glycoprotein; (D) Fraction III; (E) Fraction IV; (F) Fraction V; (G) Fraction VI. Fractions I–VI were obtained from the gel filtration experiment (Fig. 1).



Immunoelectrophoretic pattern of unfractionated pH 4.5 soluble proteins of soybean otyledons developed with a pooled antiserum to these proteins.

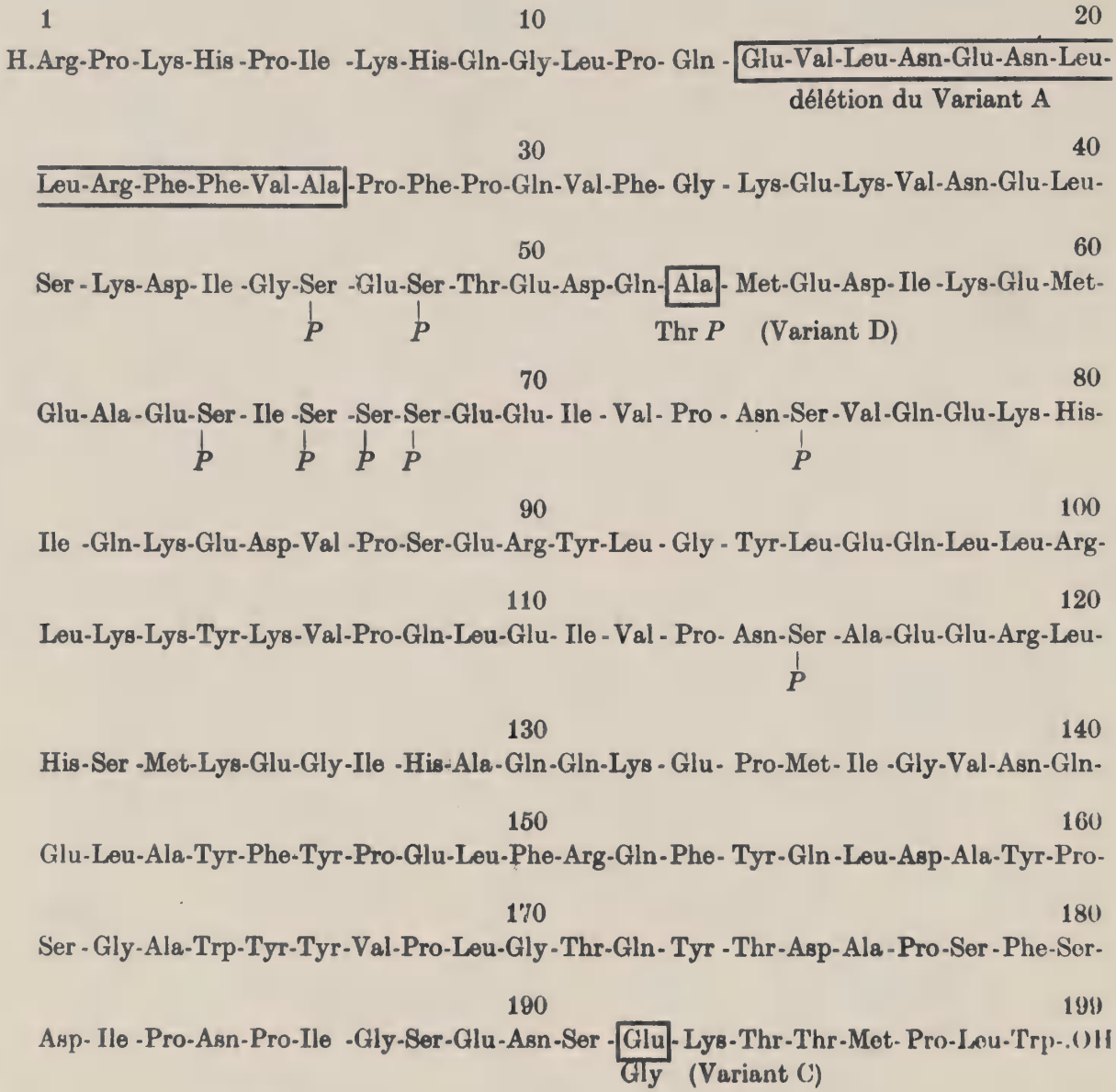


Figure 1. Primary structure of α_{s1}-casein. (Mercier, J. , European J. Biochem. 23 (1971) 41-51.)

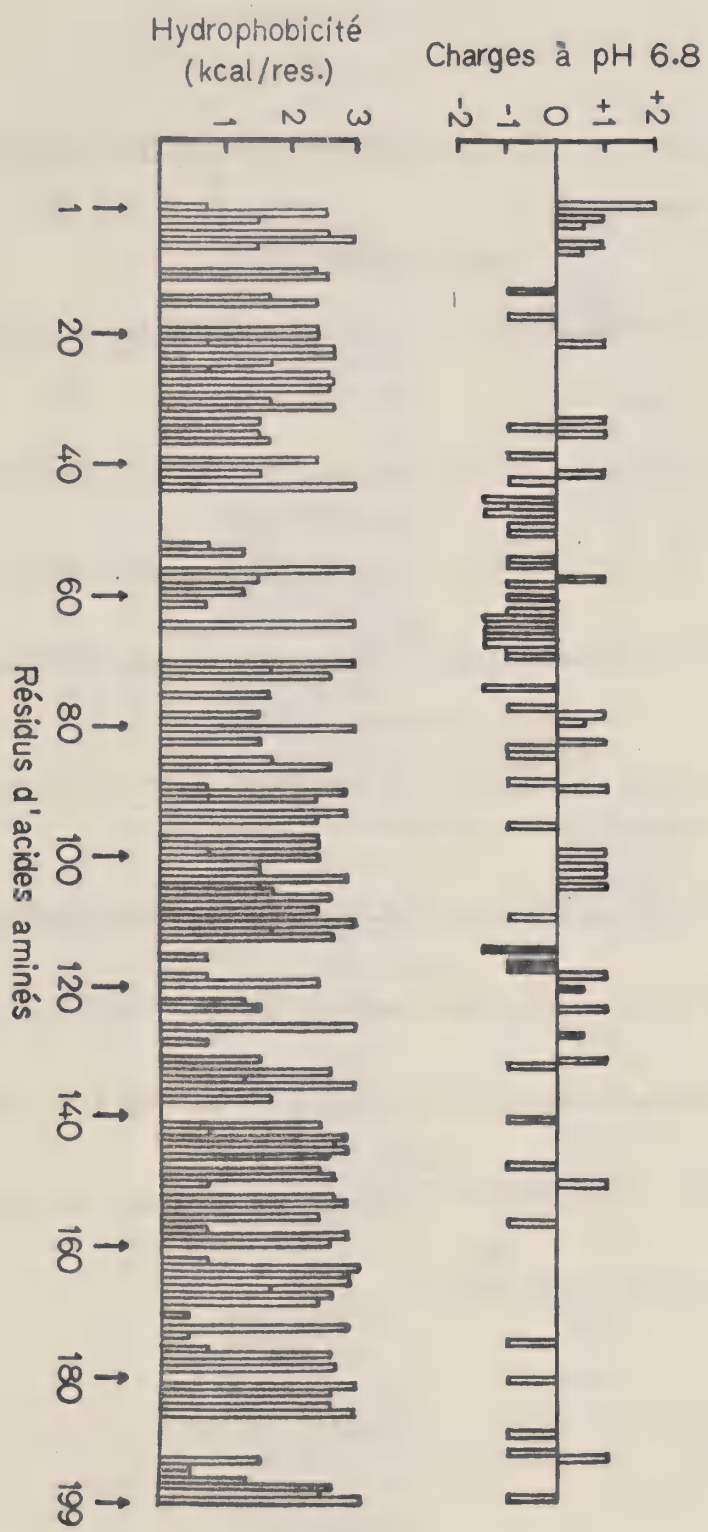


Figure 2. Diagram of the hydrophobic and hydrophilic amino acids of α_{s1}-casein. (after Mercier,J.)

Figure 3. Primary structure of β-casein. (Ribadeau-Dumas, B., European J. Biochem. 25 (1972) 505-514.)

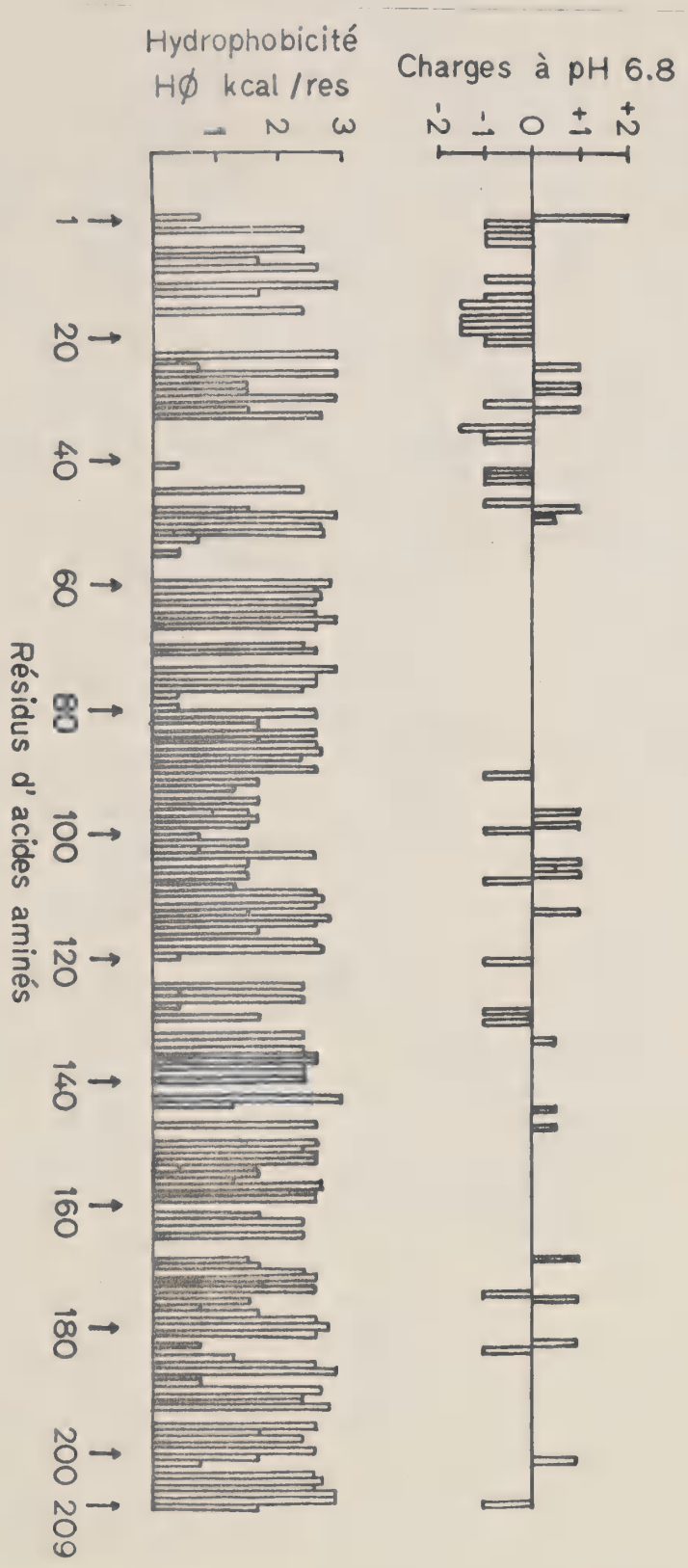
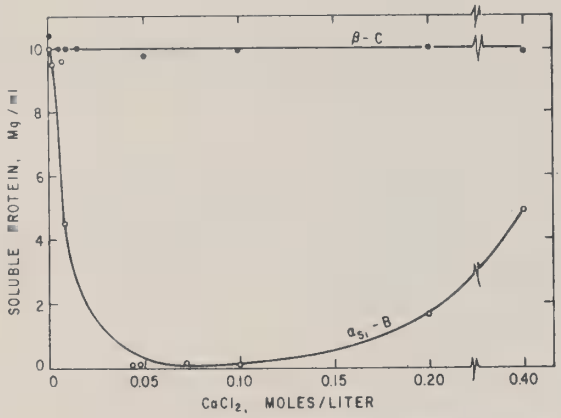
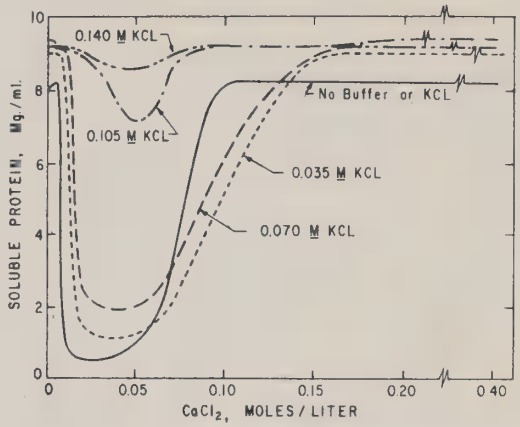


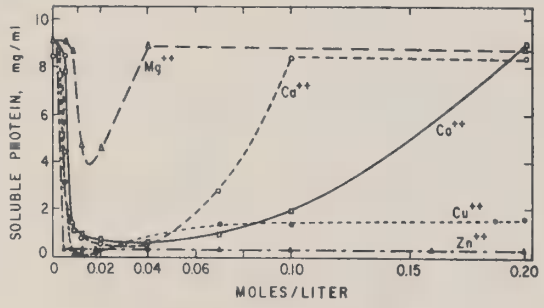
Figure 4. Diagram of the hydrophobic and hydorphilic amino acids of β-casein. (after Ribadeau-Dumas, B.)



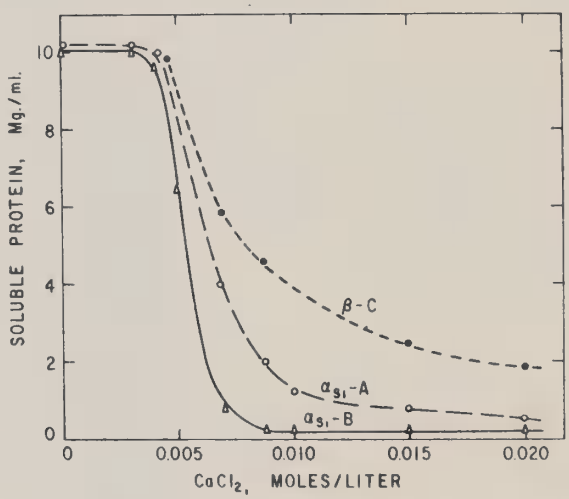
. Solubility at 1 C of calcium α_{s1}-B caseinate and calcium β-caseinate as a function of increasing $CaCl_2$ concentration at 1 C.



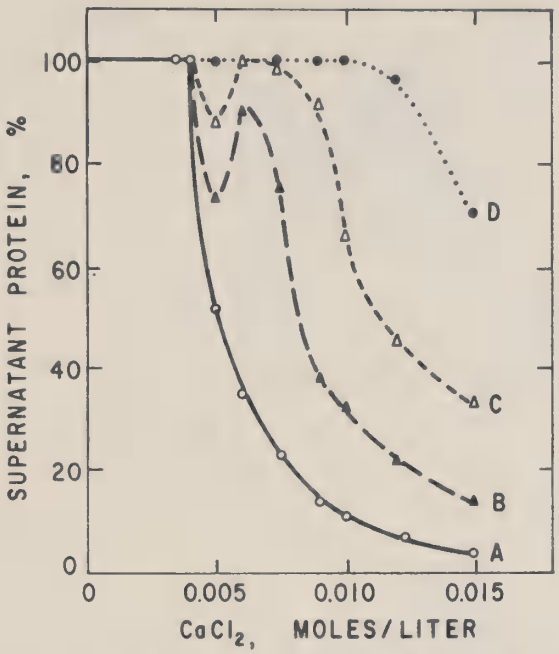
Solubility at 1 C of calcium α_{s1}-A caseinates as a function of increasing $CaCl_2$ and KCl concentrations.



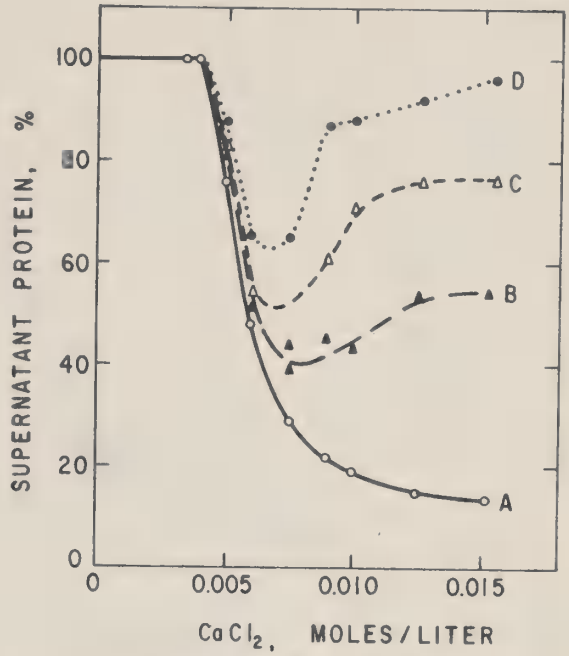
Solubility at 1 C of salts of α_{s1}-A caseinates as a function of increasing salt concentration.



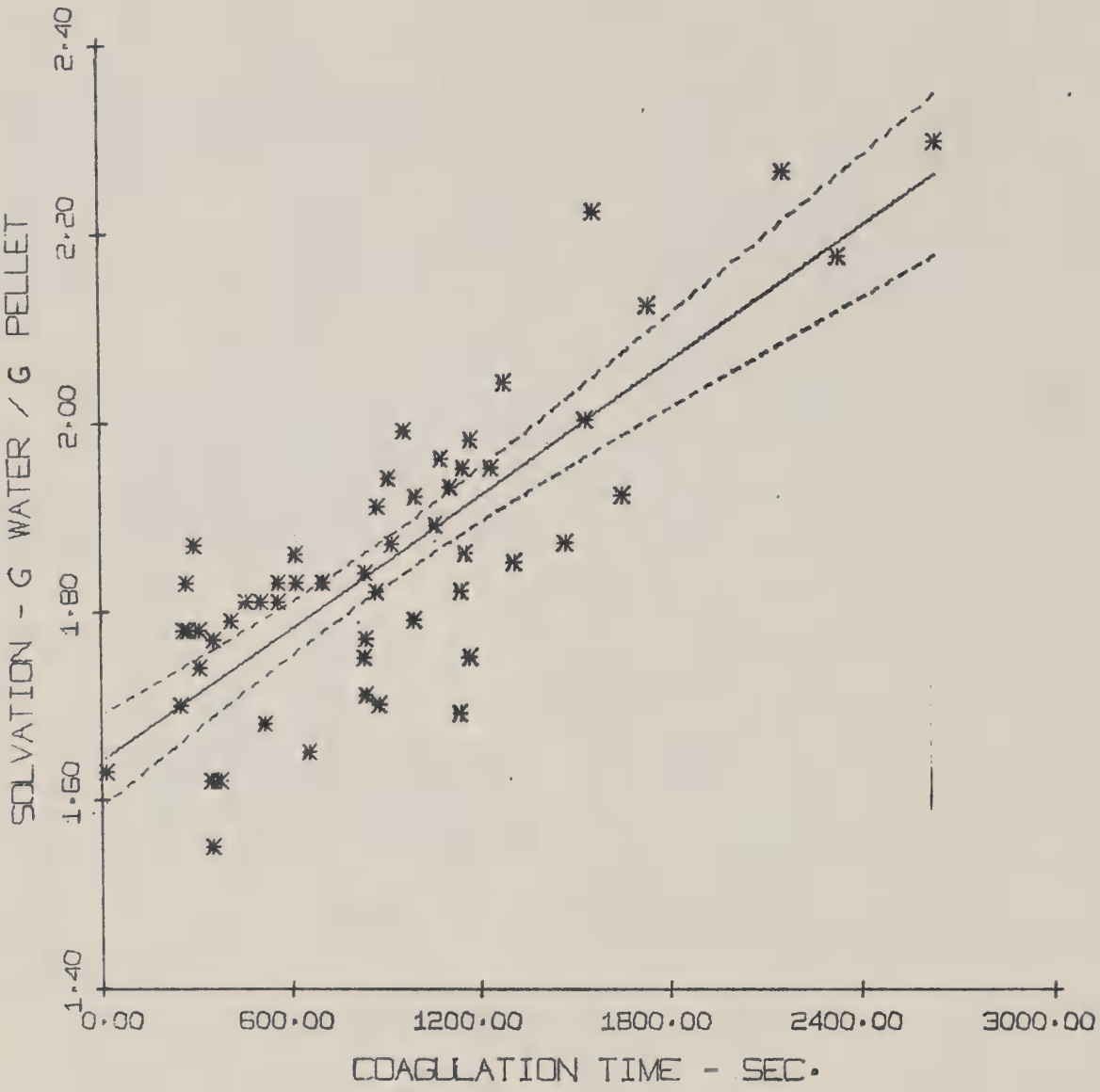
Solubility at 37 C of the calcium salts of α_{s1}-caseins A and B, and β-casein C as a function of increasing $CaCl_2$ concentration. Solutions buffered at pH 7.0, 0.01 M imidazole-HCl.



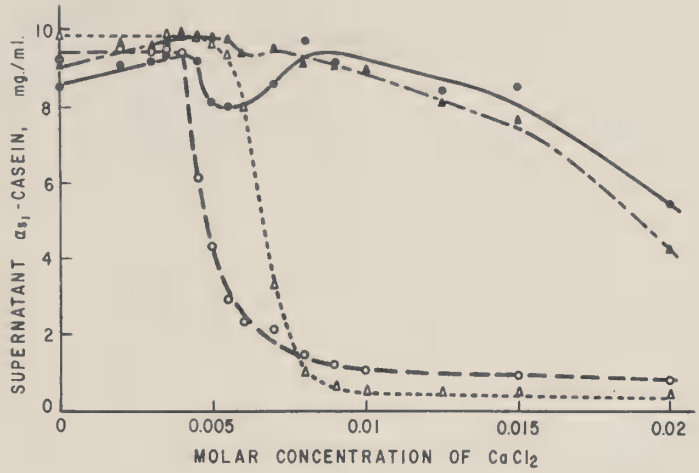
Supernatant protein at 37 C resulting from the increment addition of $CaCl_2$ to A, α_{s1}-casein B, no κ-casein; B, α_{s1}-B + κ-casein, 40:1; C, α_{s1}-B + κ-casein, 20:1; and D, α_{s1}-B + κ-casein, 10:1. Solutions buffered at pH 7.0, 0.01 M imidazole-HCl. Initial protein = 4 mg/ml.



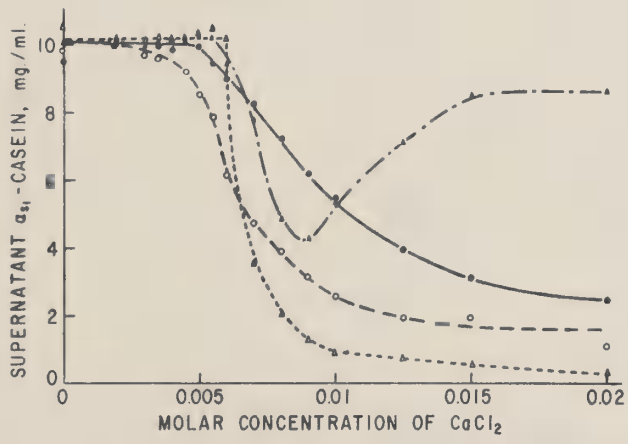
Supernatant protein at 37 C resulting from the increment addition of $CaCl_2$ to A, α_{s1}-A, no κ-casein; B, α_{s1}-A + κ-casein, 40:1; C, α_{s1}-A + κ-casein, 20:1; and D, α_{s1}-A + κ-casein, 10:1. Solutions buffered at pH 7.0, 0.01 M imidazole-HCl. Initial protein = 4 mg/ml.



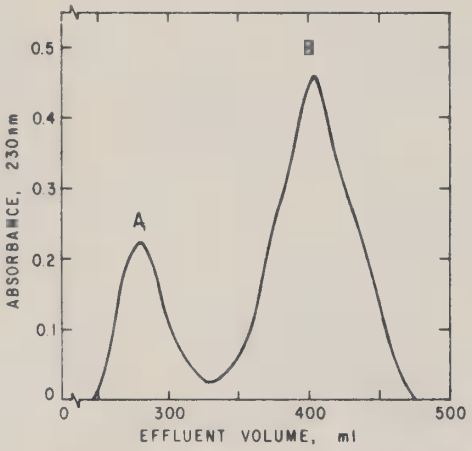
Plot of solvation values (grams water/gram pellet) versus heat coagulation time in seconds. Dotted lines represent 95% confidence limits.

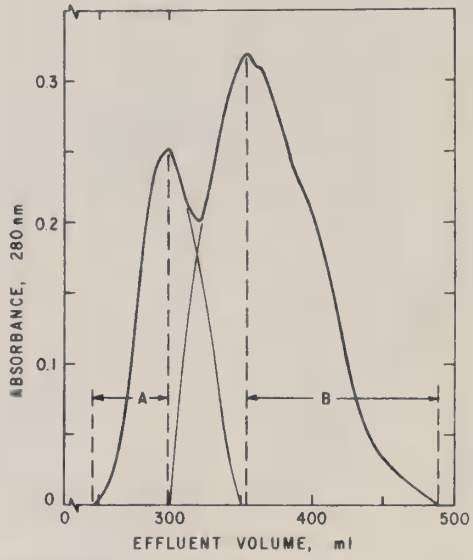


Effect of κ-casein on the solubility of α_{s1}-casein at 37° as a function of increasing $CaCl_2$ concentration in the absence of KCl. The solutions contained 0.01 M imidazole-HCl buffer (pH 7.0) and, initially, 10 mg/ml of α_{s1}-casein (native or dephosphorylated) with or without κ-casein (1.25 mg/ml). (▲) α_{s1}–κ-Casein; (●) dephosphorylated α_{s1}–κ-casein; (△) α_{s1}-casein, no κ-casein; (○) dephosphorylated α_{s1}-casein, no κ-casein.



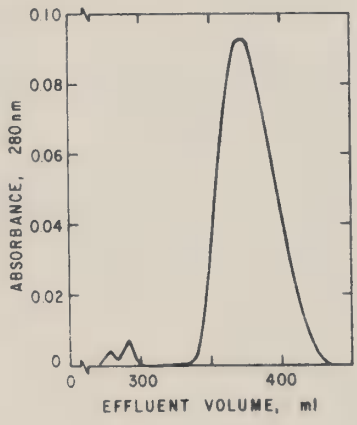
: Effect of κ-casein on the solubility of α_{s1}-casein at 37° as a function of increasing $CaCl_2$ concentration in the presence of 0.07 M KCl. The solutions contained 0.01 M imidazole-HCl buffer (pH 7.0) and, initially, 10 mg/ml of α_{s1}-casein (native or dephosphorylated) with or without κ-casein (1.25 mg/ml). (▲) α_{s1}-κ-Casein; (●) dephosphorylated α_{s1} κ-casein; (△) α_{s1}-casein, no κ-casein; (○) dephosphorylated α_{s1}-casein, no κ-casein.

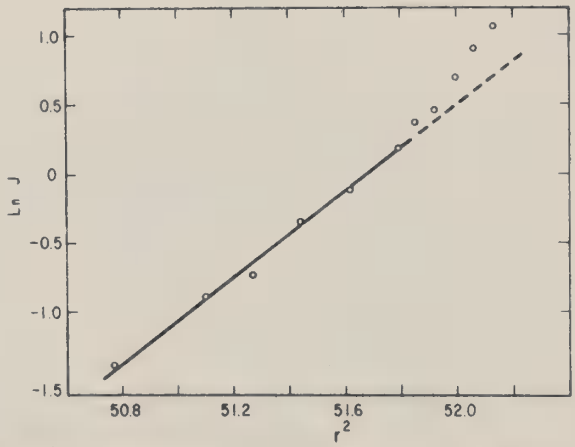




. Gel chromatography of whole acid casein. 10 mg protein in 1 ml was applied to the top of the column. V_0 = 194 ml, V_t = 465 ml.

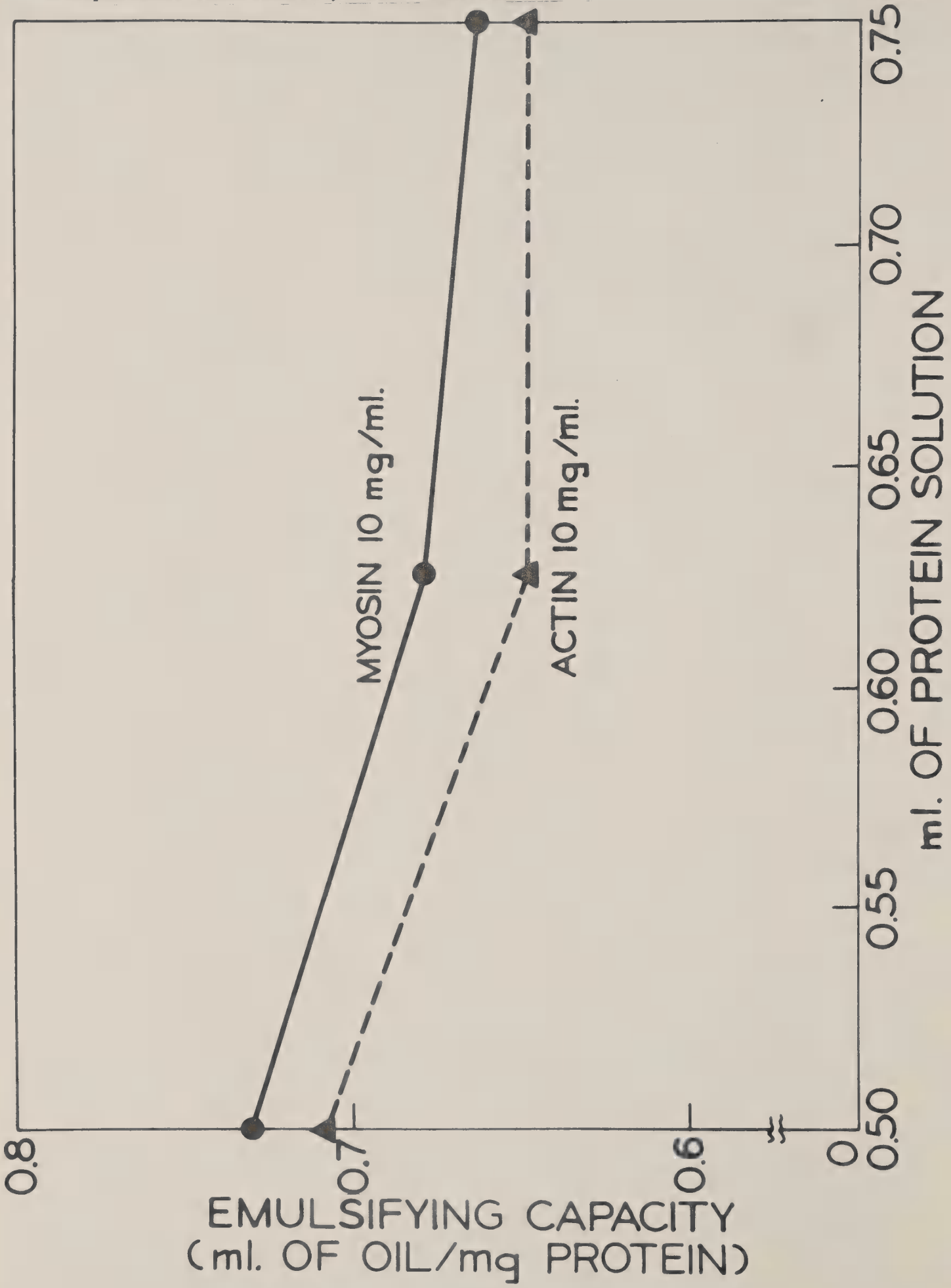
Gel chromatography of whole acid casein. 40 mg protein in 2 ml was applied to the top of the column. V_0 = 194 ml, V_t = 465 ml.

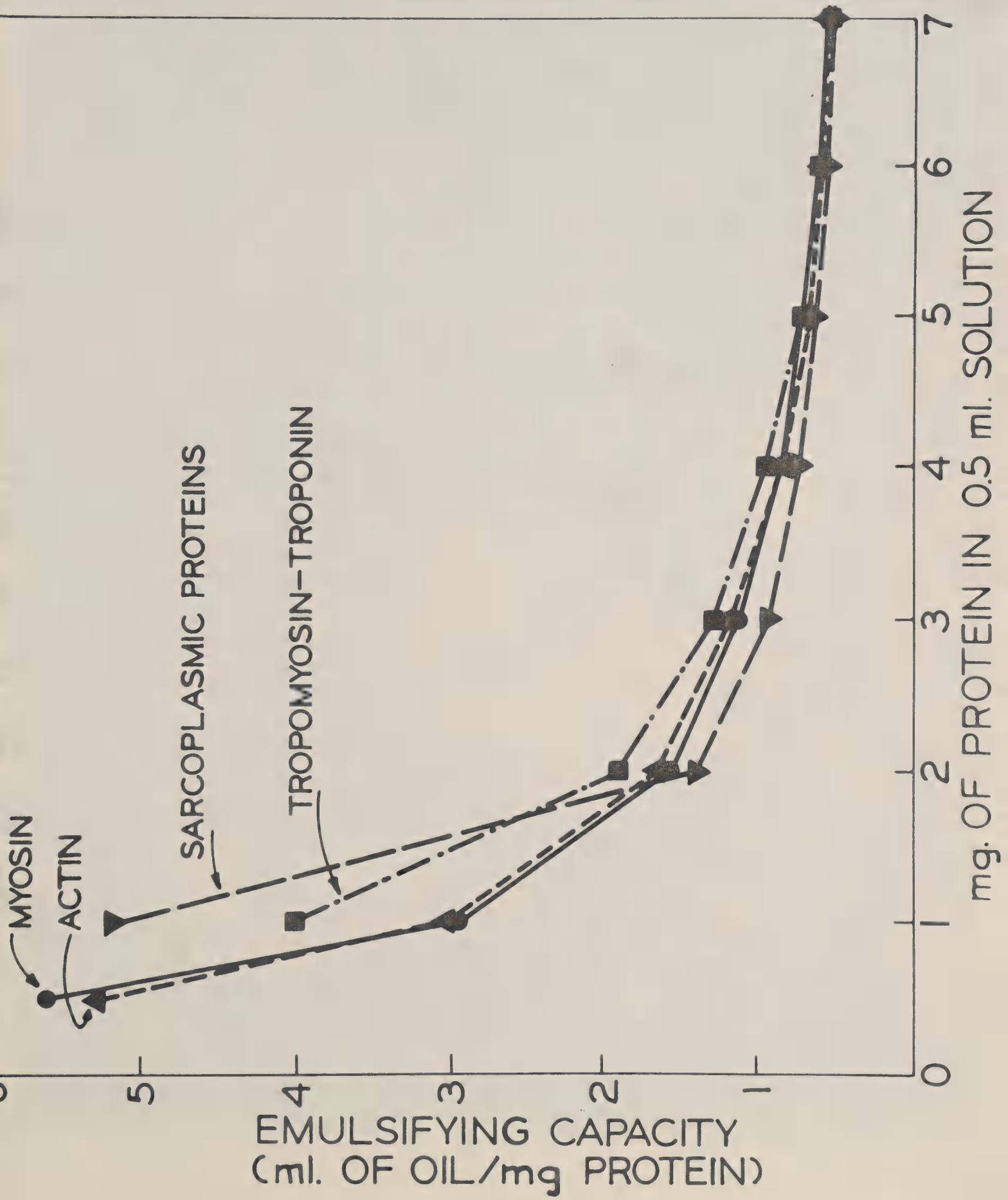


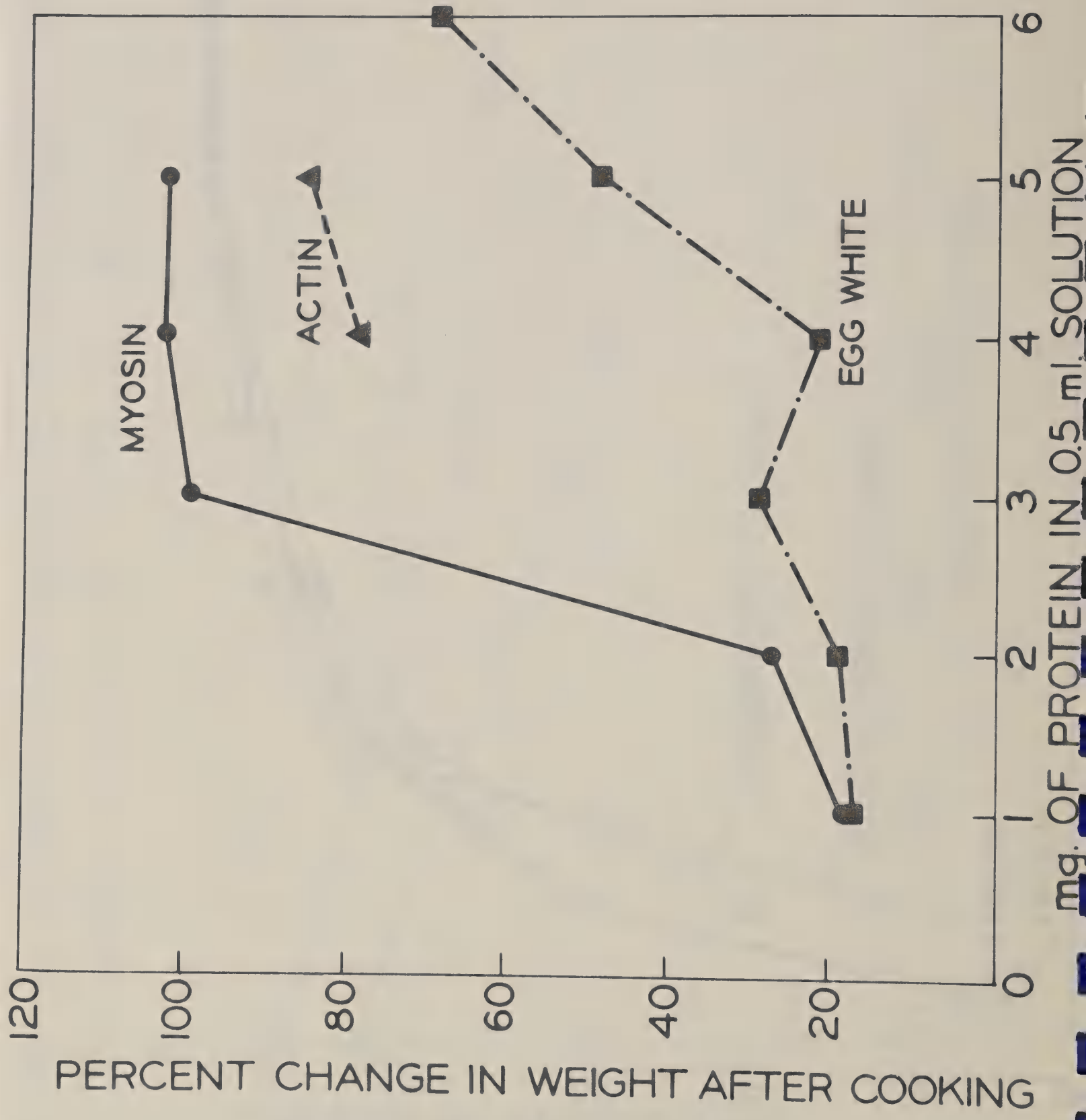


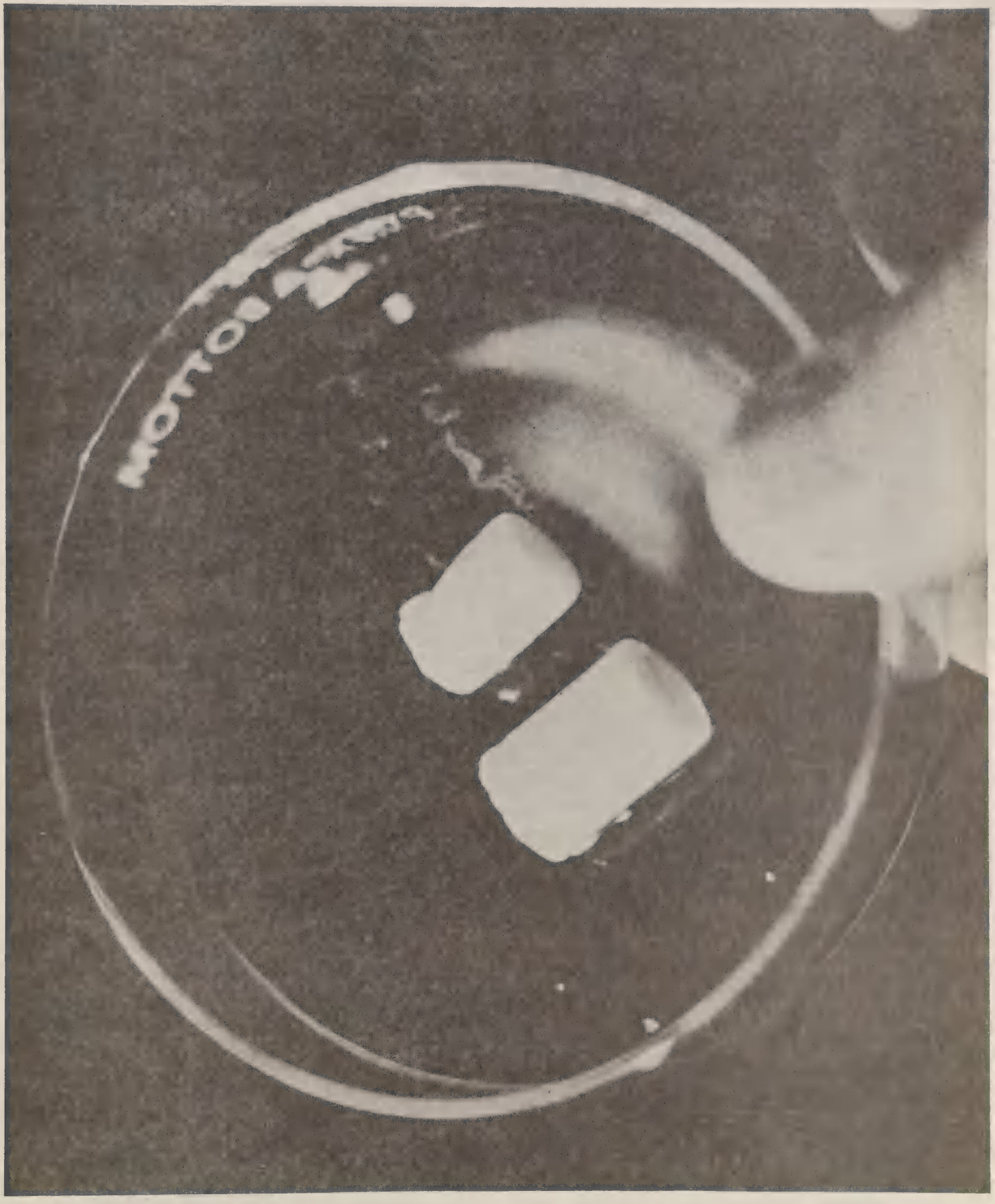
Gel chromatography of first cycle casein. 10 mg protein in 1 ml was applied to the top of the column. V_0 = 175 ml, V_t = 437 ml.

Plot of ln j against r^2 from equilibrium sedimentation of first cycle casein at a rotor speed of 9 945 rev./min, 27.5 °C and initial protein concentration of 0.25 mg/ml. The molecular weight was calculated according to the method of Yphantis[14].


MYOSIN 10 mg/ml.
ACTIN 10 mg/ml.
0.75
0.70
0.65
0.60
0.55
0.50
ml. OF PROTEIN SOLUTION
0.8
0.7
0.6
0
EMULSIFYING CAPACITY
(ml. OF OIL/mg PROTEIN)


MYOSIN
ACTIN
SARCOPLASMIC PROTEINS
TROPOMYOSIN-TROPONIN
0
1
2
3
4
5
6
7
mg. OF PROTEIN IN 0.5 ml. SOLUTION
5
4
3
2
1
EMULSIFYING CAPACITY
(ml. OF OIL/mg PROTEIN)


MYOSIN
ACTIN
EGG WHITE
120
100
80
60
40
20
0
1
2
3
4
5
6
PERCENT CHANGE IN WEIGHT AFTER COOKING
mg. OF PROTEIN IN 0.5 ml. SOLUTION



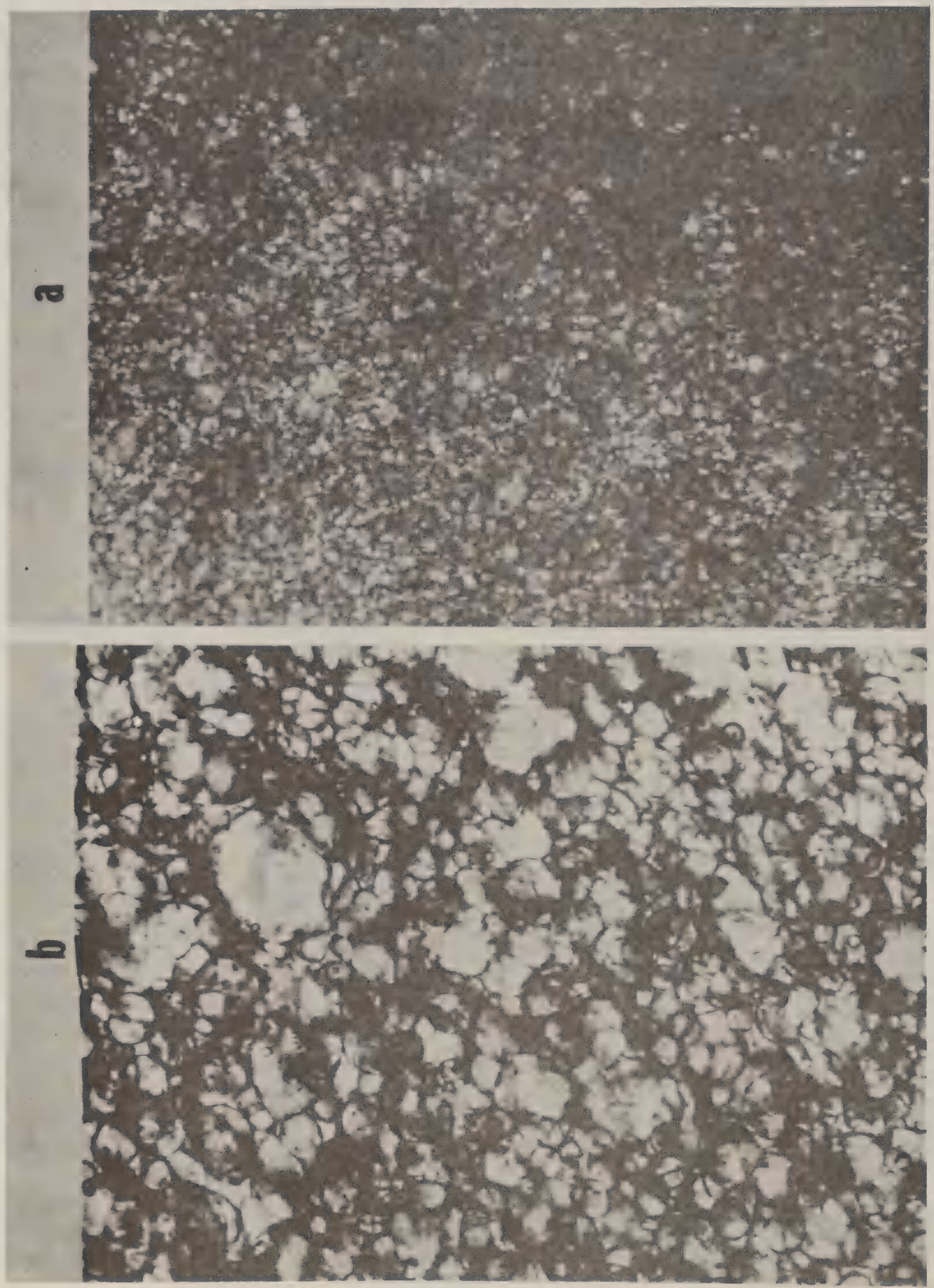

a
b

FIGURE 6

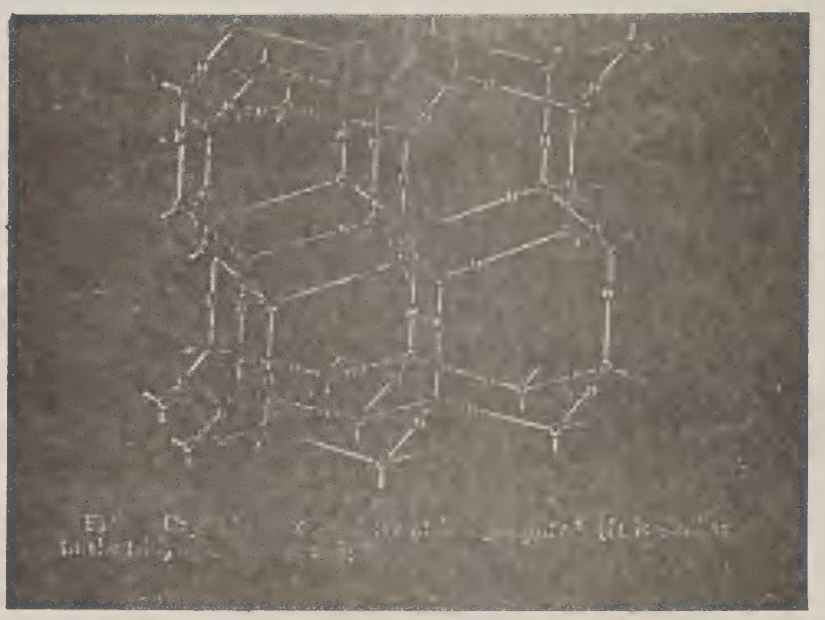


Fig. 1

Fig. 2

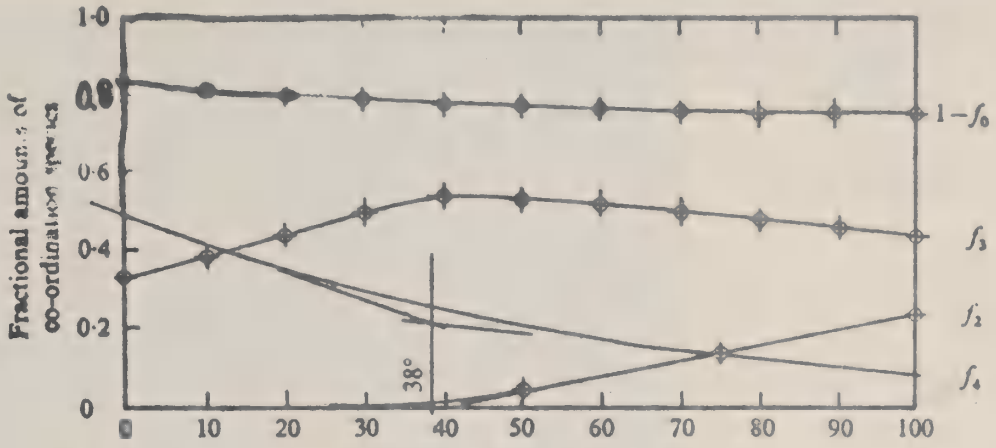


Fig. 3. Temperature dependence of the various coordination species in water computed on the basis of Walrafen's (1966) Raman intensity data

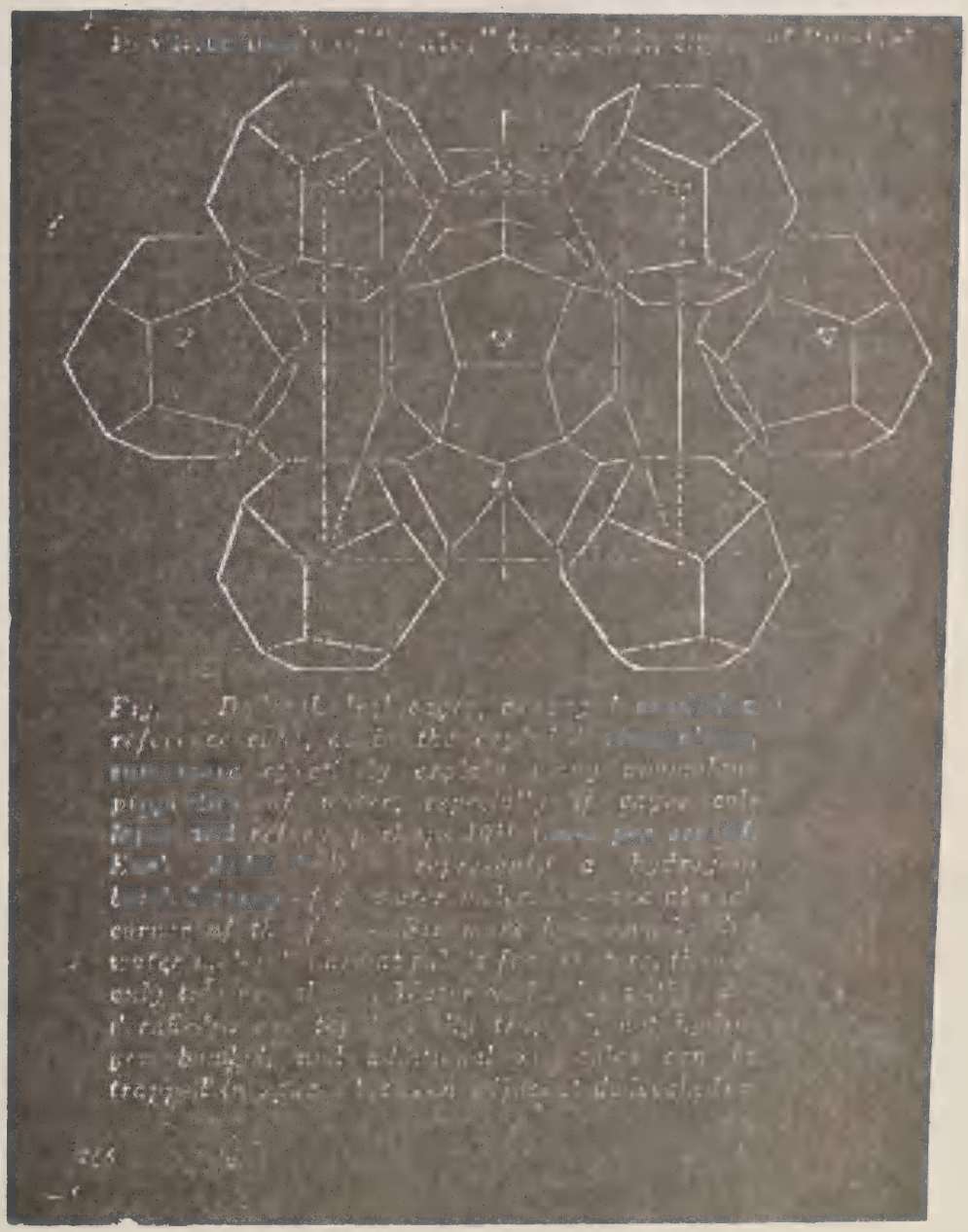
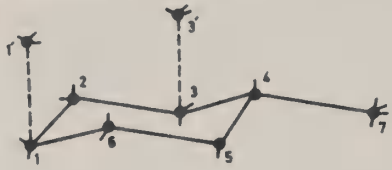


Figure 4

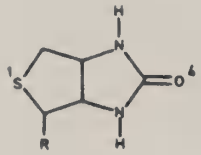
Correlation between oxygen spacings in organic molecules and in the ice lattice. Numbers in the formula indicate interaction points with the correspondingly numbered oxygens in the ice segment.

FIGURE 5

Model – ice

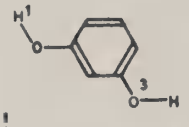


Biotin

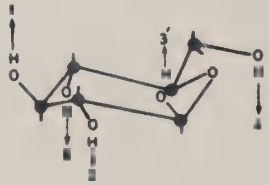


1,4-Quinone

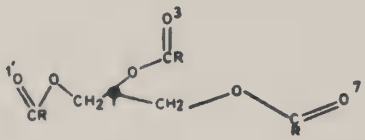
m-Dihydroxybenzene



β-D-Glucose



Triglyceride



Molecule, Reference	Repeat in Direction of Best Fit, A	Number of Water Repeats	% Deviation From Fit, Based on	
			4.74 A	4.52 A
Collagen (51)	28.6 Axial	6	+1	+5
DNA (32)	34 Axial	7	+2	+7
Feather keratin (53)	23.6 Axial (quasi-repeat*)	5	0	+4
also observed by X-ray diff	18.9 Axial	4	0	+4
TMV (23)	23 Axial (quasi-repeat*)	5	−3	+2
Cross-β protein (24)	4.65 Axial	1	−2	+3
β-Protein parallel-chain pleated sheet (47)	4.73 Perpendicular to fiber	1	0	+5
β-Protein antiparallel-chain pleated sheet (47)	9.46 Perpendicular to fiber	2	0	+5
chitin (14)	4.69	1	−1	+4
apatite (49)	9.43	2	0	+5
gramicidin S, etamycin, actinomycin, circulin, staphylomycin (57, 58)	4.8 (model)	1 (fit in hexagonal pattern)	~+1	~+6
repeat in myelin (52)	4.7		~−1	~+4

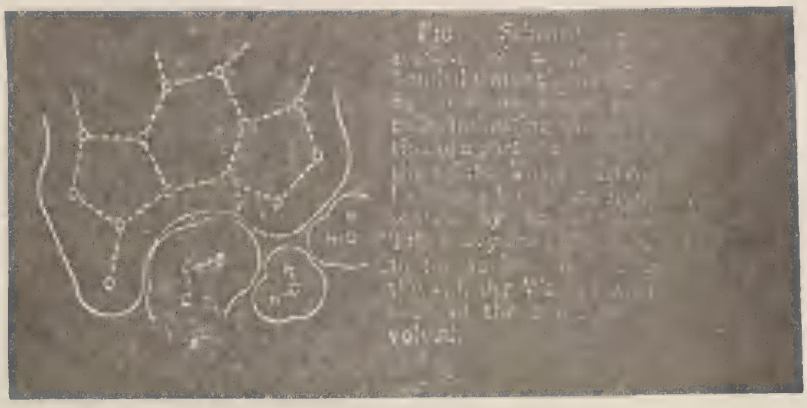
Figure 6

Figure 7

FIGURE 8

Temperature (°C)	Cluster size	Cluster collision frequency	Fraction of water molecules for the following No. of H bonds per molecule					Fraction of hydrogen bonds unbroken
			0	1	2	3	4	
0	10.0							
10	7.5							
20								
30								
40								
50								
60								
70								
80								
90								
100								

FIGURE 9



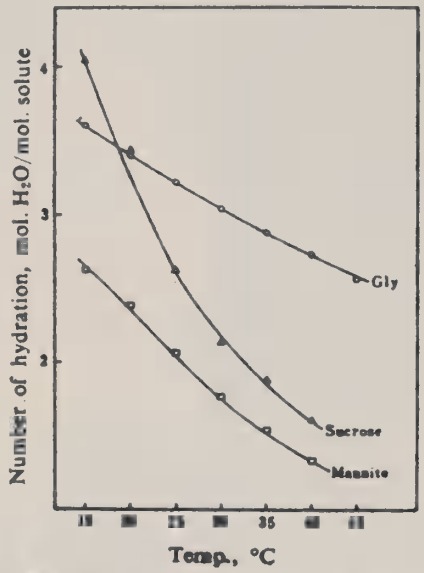


Fig. Numbers of hydration and their temperature dependence.

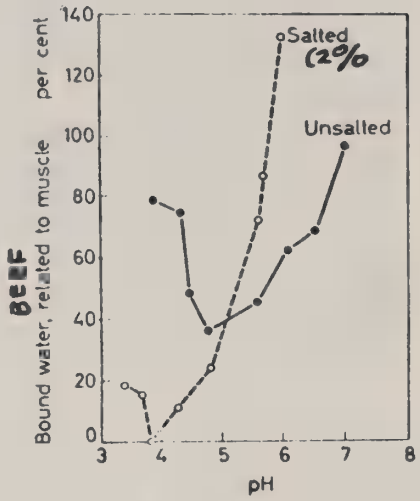


FIGURE 11

FIGURE 13

WATER ACTIVITY

1. Microorganism growth
2. Moisture equilibria
3. Reaction kinetics
4. Physiological function
5. Hydration and structure
6. Texture

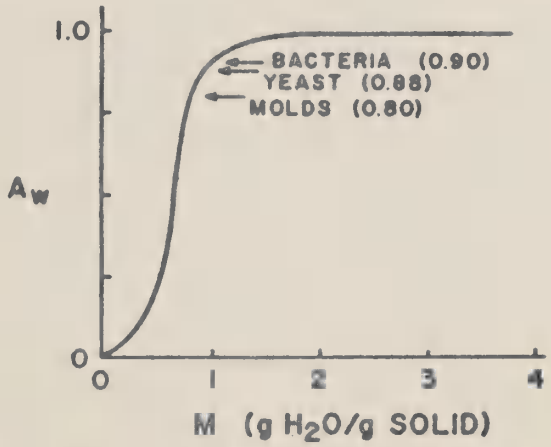


FIGURE 14 Typical water sorption isotherm (water activity A_w vs. moisture content) for a food showing growth limits of microorganisms.

FIGURE 15

Molecular Weight Effect

Polyethylene Gycol	Molality	Ideal A_w	Measured A_w
200	4.25	0.925	0.915
400	4.25	0.925	0.870
600	4.25	0.925	0.838
1000	4.25	0.925	0.680
6000	0.17	0.997	0.945
17500	0.006	0.999	0.955

FIGURE 16

EFFECTS OF GELATIN AND AGAR ON NMR SPECTRA OF D_2O AND H_2O

Concentration of gelatin or agar	Decrease in NMR peak height of D_2O		Line width of NMR spectrum of H_2O	
%	%		Hz	
	Gelatin	Agar	Gelatin	Agar
0			1.6	1.6
1			--	5.0
3	--	55	--	16.5
5	7	67	1.6	--
10	9	88	--	50.0

FIGURE 17

Pressure Effect

$$\mu = \mu o + \ln a_w$$

$$= \mu - \mu o = \int \upsilon m \, dp$$

$$a = {}_e^{\frac{\Delta P \, \upsilon m}{RT}}$$

u	$\mathring{A}$	$+a_w$	$-a_w$
0.1	1000	1.0	0.99
0.01	100	1.1	0.91
0.001	10	2.6	0.89

FIGURE 18

Surface Tension

$$d\gamma = -RT \; \Gamma_c \, d\left[\ln a_c\right]$$

As decrease solute $a_c \downarrow$ $a_w \uparrow$

surface tension increases

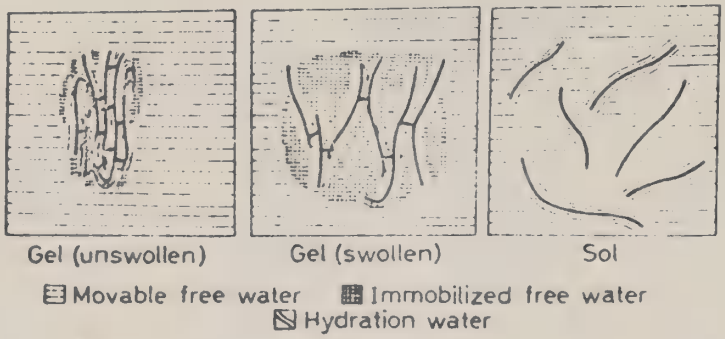


Figure Influence of cross-linking on swelling of a macromolecular system

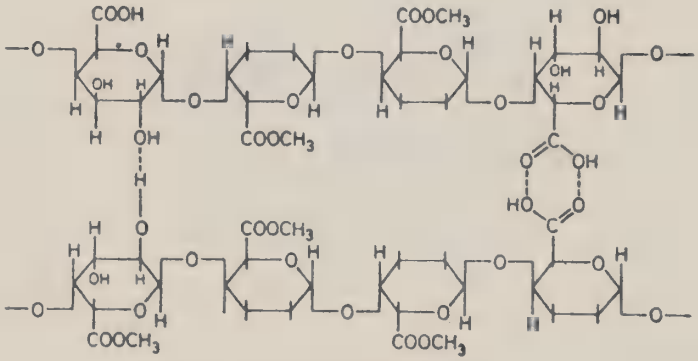


Figure 3. Secondary-valence gel of pectin[8]

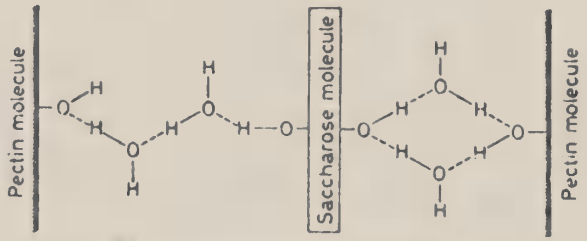


Figure Presumed structure of a pectin sugar gel[9]

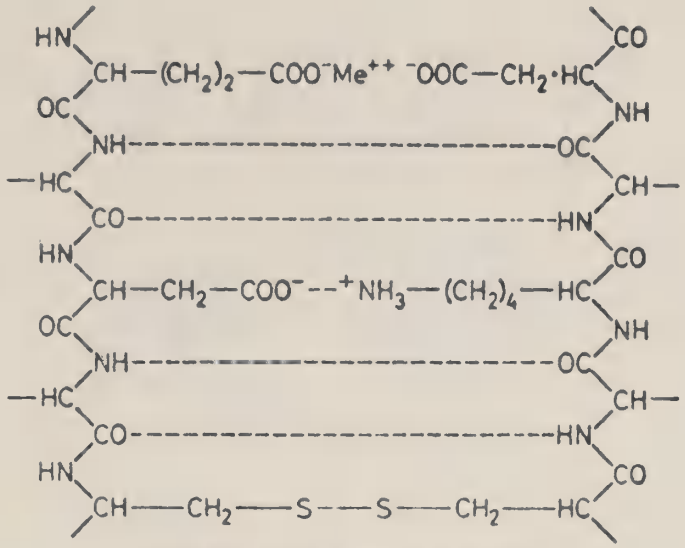


Figure 21 Cross-linkages in proteins

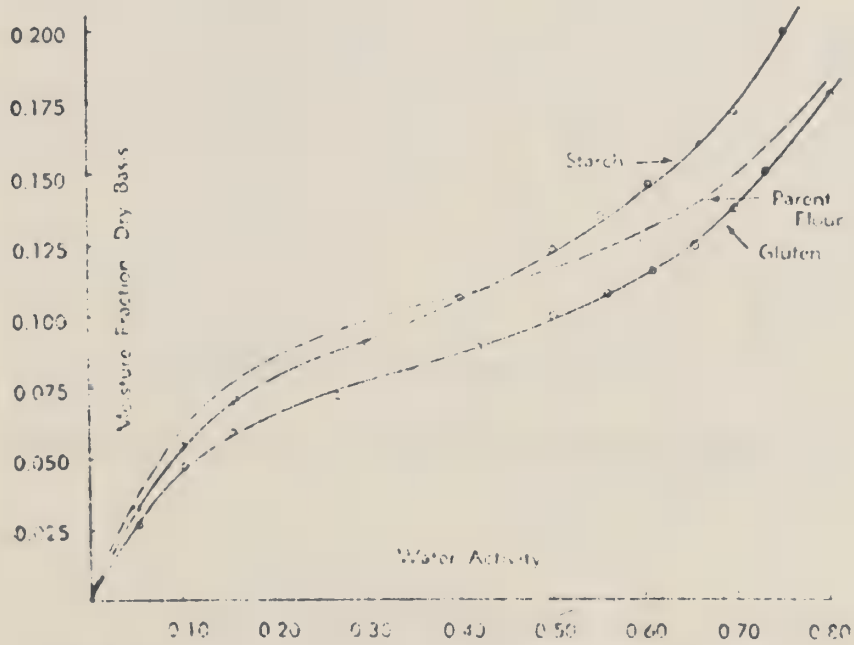


Fig. 22 Moisture adsorption isotherms at 70°F for a flour and for the starch and gluten fractionated thereform.

FIGURE 23

STARCH FUNCTIONALITY

Mean diameter	Specific solid surface	Alkaline water retention	Apparent viscosity	Cake volume	Starch damage
microns	m^2/g	cc	centipoise	cc	%
24.2	0.107	64.4	62	1225	5.8
21.3	0.117	71.1	65	1360	6.7
19.6	0.142	72.7	62	1400	6.6
18.4	0.164	81.1	72	1450	8.7

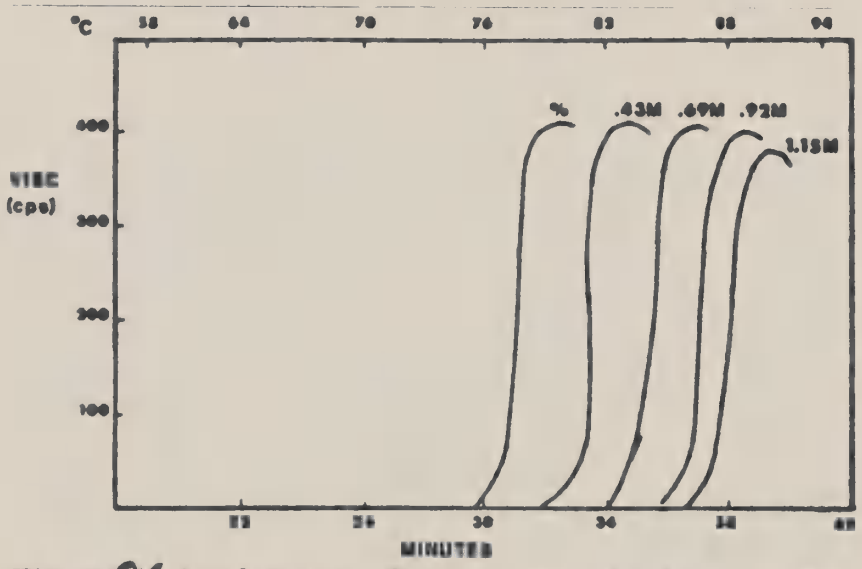


Figure 24. Amylograms of waxy corn starch at various sucrose concentrations.

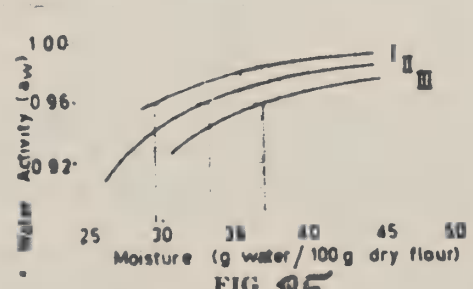


FIG. 25

Portions of the adsorption isotherms at 35°C for doughs of different composition.

Solute addition to standard formula: I. nil; II. 23 g sucrose; III. 23 g dextrose.

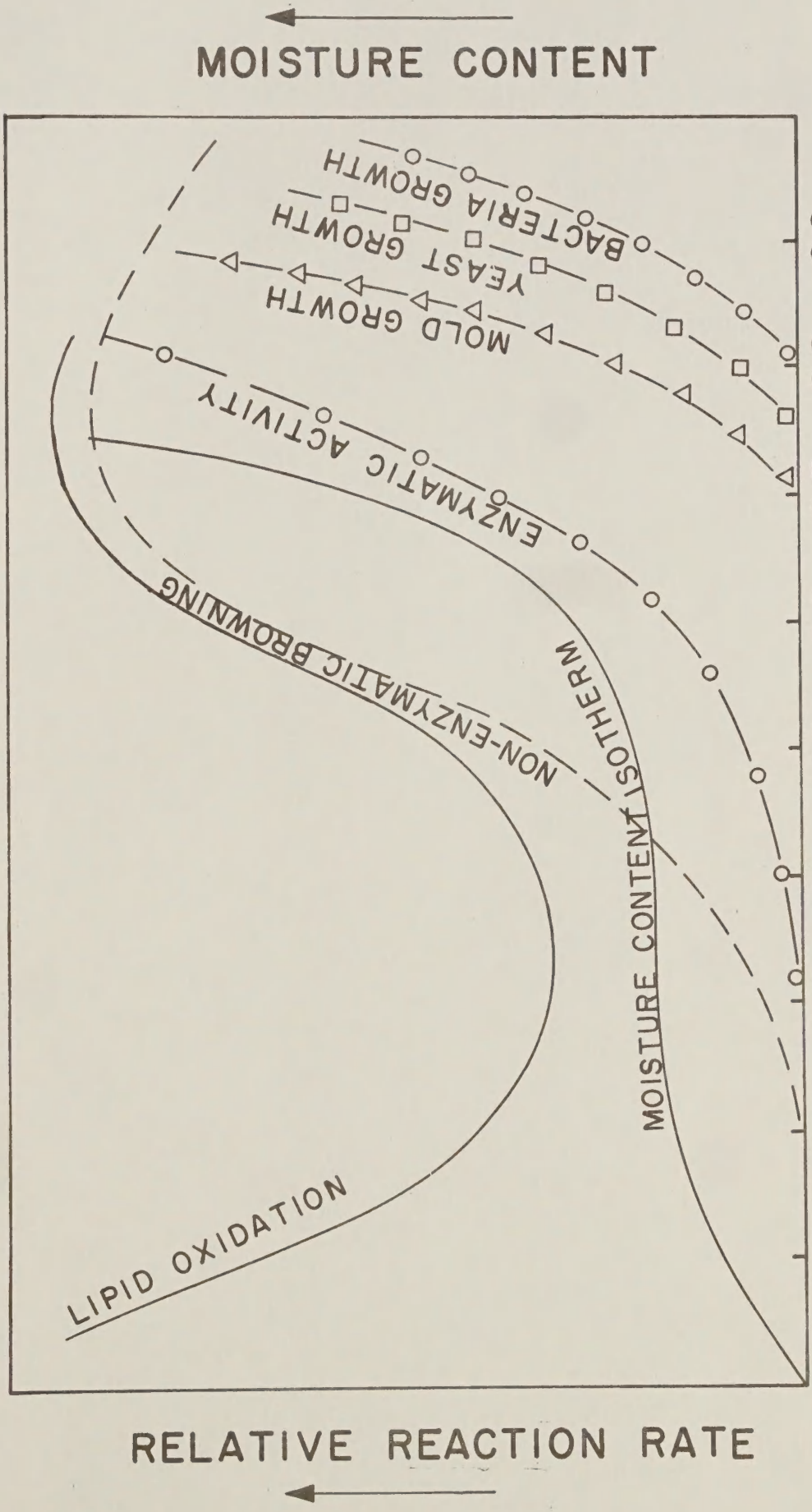

MOISTURE CONTENT
BACTERIA GROWTH
YEAST GROWTH
MOLD GROWTH
ENZYMATIC ACTIVITY
NON-ENZYMATIC BROWNING
MOISTURE CONTENT ISOTHERM
LIPID OXIDATION
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
WATER ACTIVITY
RELATIVE REACTION RATE

Figure 26